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(54) Title: ASSAYS FOR G–PROTEIN–LINKED RECEPTORS

(57) Abstract

Chimeric polypeptides derived from the Gα subunits of various G–proteins, and methods of using such chimeric polypeptides in therapy and in screening potential therapeutic agents.

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ASSAYS FOR G-PROTEIN-LINKED RECEPTORS

The field of the invention is GTP-binding proteins
5   and the receptors to which they link.

Background of the Invention

Of all the known membrane signal transducers,
heteromeric GTP-binding proteins (G proteins) are the
best characterized and the most versatile. They elicit
10 biological functions which include hormone signalling,
neurotransmission, chemotaxis, and perception of light,
smell, and taste. G proteins couple to various cell
surface receptors (G-linked receptors) and activate
various intracellular effectors. Each G protein is made
15 up of a $G\alpha$ subunit and a $G\beta\gamma$ subunit. The specificity of
G proteins' coupling to receptors and downstream
signalling molecules is conferred by the various $G\alpha$
subunits. The $G\alpha$ molecules are classified into two
categories: one is a class of sensory-organ-specific G
20 proteins (e.g., $G\alpha_t$, $G\alpha_{olf}$, and $G\alpha_{gust}$), and the other is a
less tissue-specific class consisting of $G\alpha_s$, the $G\alpha_i$
family (i.e., $G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$, $G\alpha_{o1}$, $G\alpha_{o2}$, and $G\alpha_z$), the
$G\alpha_{12}$ family (i.e., $G\alpha_{12}$ and $G\alpha_{13}$), and the $G\alpha_q$ family
(i.e, $G\alpha_q$, $G\alpha_{11}$, $G\alpha_{14}$, and $G\alpha_{16}$). It is likely that more
25 members of each class will be discovered.

Experiments using recombinant $G\alpha$ chimeric
molecules which have some peptide sequence derived from
one type of $G\alpha$ and additional sequence from another type
of $G\alpha$ (e.g., $G\alpha_{13}/\alpha_z$, $G\alpha_q/\alpha_{i2}$, and $G\alpha_{i2}/\alpha_{i1}$) have helped
30 distinguish the receptor-specifying portions of these
particular $G\alpha$ molecules from their effector portions
(Conklin et al., Nature 363: 274-276, 1993; Voyno-
Yasenetskaya et al., J. Biol. Chem. 269: 4721-4724, 1994;
Law et al., Mol. Pharmacol. 45: 587-590, 1994).

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Furthermore, using a scanning mutagenesis approach, Berlot and Bourne (Cell 68: 911-922, 1992) have identified the shortest linear stretch (residues 236-356) in $G\alpha_s$ essential for $G\alpha_s$'s interaction with its effector,
5 adenylyl cyclase.

Summary of the Invention

Applicants have established a $G\alpha_s$-based chimeric system for identifying the $G\alpha$ subunit of a G protein to which a given G-linked receptor couples. A series of
10 $G\alpha_s/\alpha_x$ chimeras ($G\alpha_x$: any $G\alpha$ subunit except $G\alpha_s$) can be made with a first amino acid sequence corresponding to a region of $G\alpha_s$ (SEQ ID NO:21) encompassing $G\alpha_s$'s residues 236-356, followed by a second amino acid sequence 4-30 amino acids long and corresponding to a segment of $G\alpha_x$,
15 which segment ends at (and includes) $G\alpha_x$'s C-terminal residue. The first amino acid sequence should contain the effector portion of $G\alpha_s$, and preferably will contain residues 1-389 of SEQ ID NO:21. The second amino acid sequence should contain the receptor-coupling portion of
20 $G\alpha_x$, and preferably is 4 or 5 amino acids in length (e.g., as represented by SEQ ID NOs:22-30). Once the chimera is coupled to a $G\alpha_x$-coupled receptor via the $G\alpha_x$ portion of the chimera, the chimera can transduce a signal from the receptor to adenylyl cyclase (AC) via the
25 $G\alpha_s$ portion of the chimera, resulting in an increase in cyclic AMP (cAMP) in the cell. Since the normal signalling pathway of non-chimeric $G\alpha_x$ does not involve AC, stimulation of the $G\alpha_x$-coupled receptor in the absence of the $G\alpha_s/\alpha_x$ chimera does not result in an
30 increase in cellular cAMP.

In the present method, two identical samples of cells are provided, wherein the cells co-express a given G-linked receptor and a given $G\alpha_s/\alpha_x$ chimera. The second sample of cells is contacted with a ligand of the G-

linked receptor. AC activity, as manisfested by the rate
of cAMP formation, is measured in both samples of cells.
A significant increase in cAMP formation in the second
sample as compared to the first sample indicates that

5 that particular $G\alpha_x$ can couple to the receptor.

Cells co-expressing a given G-linked receptor and
a given $G\alpha_s/\alpha_x$ chimera can be established by introducing
into the cells a recombinant nucleic acid construct
permitting expression of the receptor and a second

10 recombinant nucleic acid construct permitting expression
of the chimera. By "recombinant" is meant that the
nucleic acid (or polypeptide) molecule is the product of
artificial genetic manipulation.

As used herein, a $G\alpha_s/\alpha_x$ chimera is a polypeptide

15 which includes the AC-coupling portion (e.g., amino acid
residues 236-356) of $G\alpha_s$ (SEQ ID NO:21) as well as the
receptor-coupling portion of $G\alpha_x$. The receptor-coupling
portion can be 4-30 amino acids long and usually
corresponds to the extreme C-terminal region of $G\alpha_x$. The

20 chimeric polypeptide can also include an additional
peptide sequence such as one that serves as an epitope
tag, so long as the additional sequence does not
interfere with the functioning of the chimera. By "G-
linked receptor" is meant any naturally occurring cell

25 surface receptor, or any functional recombinant variant
thereof, that couples to a G protein. By "significant"
is meant that the two values in comparison have a p value
of less than 0.05 in Student's t test. By "ligand" is
meant any molecule that binds and activates a receptor.

30 A ligand can be, for example, the natural, physiological
activator of the receptor (e.g., a hormone), a
biologically active analogue thereof, or an antibody
which binds to and thereby activates the receptor.

The chimeras of the invention can also be used in

35 a method of screening compounds for their ability to

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modulate the interaction between a given G-linked
receptor and the Gα (i.e., Gα_x) subunit of a non-G$_s$ G
protein known to couple to the receptor. In the method,
two identical samples of cells are provided, wherein the
5 cells co-express the G-linked receptor and a Gα_s/α_x
chimera. Both samples of cells are contacted with a
ligand of the G-linked receptor. The second sample is
additionally contacted with a test compound. cAMP
formation is then measured in both samples. A
10 significant decrease (or increase) of the cAMP level in
the second sample as compared to the first sample
indicates that the compound is capable of inhibiting (or
enhancing) the interaction between the G-linked receptor
and that particular Gα_x.

15   In this method, one can screen compounds that can
modulate the following exemplary interactions: those
between (1) Gα_i and somatostatin receptor (SSTR) type 1,
SSTR 3, insulin-like growth factor II receptor,
muscarinic acetylcholine receptor, D$_2$-dopamine receptor,
20 α_2-adrenergic receptor, adenosine receptor, thrombin
receptor, or transforming growth factor β receptor; (2)
Gα_z and SSTR1; (3) SSTR3 and either Gα_{14} or Gα_{16}; (4)
SSTR5 and either Gα_{12} or Gα_{13}; (5) Gα_o and amyloid protein
precursor (APP), transforming growth factor-β receptor,
25 γ-butyric acid receptor, muscarinic acetylcholine
receptor, adenosine receptor, thrombin receptor, or α_2-
adrenergic receptor; or (6) Gα_q and the T cell receptor,
PTH/PTHrP receptor, calcitonin receptor, endothelin
receptor, angiotensin receptor, platelet activating
30 factor receptor, or thromboxane A$_2$ receptor. One can
also use any constitutively active variants of these
receptors, thereby eliminating the need for contacting
the above-described cell samples with the receptors'
ligands.

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The chimeras have an inherent ability to alter the
signal-transducing output of a given G-linked receptor.
By "signal-transducing output" is meant the end result of
the signalling initiated by a liganded G-linked receptor.
5   Such an end result can be, for example, cell growth
inhibition, cell proliferation, or secretion of a
protein. To achieve this alteration, one can introduce
into a target cell a $G\alpha$ chimeric polypeptide containing
the sequence of a $G\alpha$ linking to a desirable effector, the
10 receptor-coupling region (e.g., the 4-30 residues at the
C-terminal end) of which sequence is replaced with that
of a $G\alpha$ to which the G-linked receptor normally couples.
Such a chimeric polypeptide can be employed in a method
of therapy for a condition associated with the function
15 or lack of function of that receptor in a patient's
cells. For instance, one can convert the activity of a
constitutively active mutant of amyloid protein precursor
(APP, known to couple to $G\alpha_o$) from AC-suppressing to AC-
activating by supplying to a neural cell harboring the
20 APP mutant a therapeutically effective amount of $G\alpha_s/\alpha_o$,
e.g., by genetic therapy. The $G\alpha_s/\alpha_o$ polypeptide can be
introduced into the target cell by introducing into the
cell a recombinant nucleic acid construct that permits
expression of the chimeric polypeptide. Such a construct
25 can, for instance, be derived from a herpes simplex viral
vector, or any other vector able to transfect neural
cells.
      Also within the invention is a method of improving
the tumor growth inhibition ability of somatostatin (SST)
30 or its known biologically active analogues. SST is known
to inhibit growth of certain tumors, presumably by
binding to SST receptors (SSTR) on the cell surface and
inhibiting cell proliferation. It has been observed that
in cancer treatments involving SST-related drugs, certain
35 tumors become resistant to the drugs after a period of

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time, presumably due to loss of SSTR5 expression on cell
surface. Expression of a recombinant SSTR5 protein in
the tumor cells can circumvent this problem. By "tumor
growth inhibition" is meant that a tumor cell is
5   prevented from proliferating or is induced to undergo
apoptosis. Somatostatin is a 14 amino acid cyclic
peptide hormone which was originally isolated from the
hypothalamus. Biologically active analogues of SST
include, but are not limited to, (1) naturally occurring
10   analogues, such as SST-28 (FEBS Lett. 282: 363-367, 1991)
and SST-25 (Gen CompEndocinol 81: 365-372, 1991); and (2)
artificial compounds, such as octreotide (New Engl. J.
Med. 334: 246-254, 1995), RC-160 (Buscail et al., PNAS
92: 1580-1584, 1995), RC-160-I and RC-160-II (Cancer Res.
15   54: 5895-5901, 1994), SMS 201-995 (Kubota et al., J.
Clin. Invest. 93: 1321-1325, 1994), and BIM-23014 (i.e.,
lanreotide) (FASEB J. 7: 1055-1060, 1993).
          Another method of inhibiting tumor growth is
useful for tumor cells the growth of which is stimulated
20   via an endogenous, hyperactive G-linked receptor. By
"endogenous" is meant that the receptor is expressed in
the cell absent any artificial genetic manipulation. By
"hyperactive" is meant that the G-linked receptor is more
active, or active for a longer period of time, than it is
25   in a normal cell. Hyperactivity of a G-linked receptor
can be caused by, for example, certain mutations in the
receptor's peptide sequence, an unusually high level of
the receptor's ligand, and/or a ligand that dissociates
from the receptor at a rate lower than normal. One can
30   then introduce into the tumor cell a $G\alpha_{12}$ or $G\alpha_{13}$ chimeric
molecule, the C-terminal 5 residues of which are replaced
with those of the $G\alpha$ that the hyperactive receptor
normally couples to. Thus, the hyperactivity of the
receptor is transduced via the $G\alpha_{12}$ or $G\alpha_{13}$ chimeric
35   molecule to downstream growth-inhibitory effectors, which

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counteract at least in part growth-stimulatory signals
normally transduced by the receptor and its cognate G
protein. Preferably, the chimera will transduce a signal
that results in apoptosis of the tumor cell. The
5 chimeric molecule can be introduced into the target cell
in vivo, *in vitro*, or *ex vivo* in a carrier such as saline
and/or liposomes. It can also be expressed by a
recombinant nucleic acid construct that has been
introduced into the cell.

10   Brief Description of the Drawings
        Fig. 1 is a schematic representation of the $G\alpha_s$
chimeras constructed in the study. "$G\alpha_s$ wt" denotes
wild-type $G\alpha_s$. Sequences of the last 5 C-terminal
residues of the chimeras are illustrated, and referred to
15 as SEQ ID NOs:22-31. These sequences are identical
between $G\alpha_{i1}$ and $G\alpha_{i2}$, between $G\alpha_{o1}$ and $G\alpha_{o2}$, and between
$G\alpha_q$ and $G\alpha_{11}$.
        Fig. 2A is a bar graph showing the effects of SST
on cholera toxin (CTX)-stimulated AC activity in cells
20 expressing SSTR3. Cells were transfected with 0.125 μg
of pCMV6-SSTR3 and 0.125 μg of pCMV6 vector. At 24 h
after transfection, cells were treated for 30 min with or
without 1 μM SST, in the presence of (1) 1 mM IBMX, or
(2) 1mM IBMX plus 250 ng/ml CTX. cAMP formation was
25 subsequently measured. All values are "means ± S.E." of
quadruplicated experiments.
        Fig. 2B & Fig. 2C are bar graphs showing the
effects of SST on cAMP formation in cells expressing a
$G\alpha_s$ chimera with (Fig. 2C) or without (Fig. 2B) SSTR3.
30 Cells were transfected with 0.125 μg of plasmid encoding
a $G\alpha_s$ chimera and 0.125 μg of either pCMV6-SSTR3 (Fig.
2C) or pCMV6 (Fig. 2B). At 24 h after transfection,
cells were treated for 30 min with (1) 1 mM IBMX, or (2)
1 mM IBMX plus 1 μM SST. cAMP formation was subsequently

measured.   AC activity levels are represented as
percentage relative to the basal AC activity level in
cells expressing $G\alpha_s/\alpha_{i1}$ alone.   All values are "means
± S.E." of quadruplicated experiments.   Similar results
5   were found at least three times for each chimera.

Fig. 2D is a bar graph converted from Fig. 2B,
showing the ratios of cAMP levels in the presence vs.
absence of SST in transfected cells.

Fig. 3A & Fig. 3B are bar graphs showing the
10   effects of SST on AC activity in cells expressing a $G\alpha_s$
chimera with (Fig. 3A) or without (Fig. 3B) SSTR3.   Cells
were transfected with 0.125 μg of plasmid encoding a $G\alpha$
chimera and either 0.125 μg of pCMV6-SSTR3 (Fig. 3A) or
pCMV6 (Fig. 3B).   Experiments were performed as described
15   in the legend for Figs. 2A and 2B.   AC activity levels
are represented as percentage relative to the basal AC
activity level in cells expressing $G\alpha_s/\alpha_q$ alone.   All
values are "means ± S.E." of quadruplicated experiments.
Similar results were found at least three times for each
20   chimera.

Fig. 3C is a bar graph showing the effects of SST
on cAMP formation in cells expressing SSTR3 and a $G\alpha_s$
chimera derived from the $G\alpha_i$ or $G\alpha_q$ family.   All the
indicated chimeras were tested in parallel.   Experiments
25   were performed as described in the legend for Figs. 2A
and 2B.   All values are "means ± S.E." of quadruplicated
experiments.   Similar results were found at least three
times for each chimera.

Fig. 3D is a bar graph converted from Fig. 3C,
30   showing the ratios of cAMP levels in the presence vs.
absence of SST in transfected cells.

Fig. 4A is a bar graph showing the stimulation of
inositol phosphate (IP) production in cells transfected
with (1) 0.125 μg of pCMV6-SSTR3, and (2) 0.125 μg of
35   plasmid encoding the intact $G\alpha_{16}$.   At 24 h after

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transfection, cells were treated for 5 min with or without 1 μM SST, and IP production was measured. For treatment with pertussis toxin (PTX), at 24 h after transfection, cells were treated with 10 ng/ml PTX for
5 3 h and with SST as described above.

Fig. 4B is a bar graph showing the stimulation of IP production in cells transfected with (1) 0.125 μg of pCMV6-SSTR3, and (2) 0.125 μg of plasmid encoding intact $G\alpha_{14}$. Experiments were performed as described in
10 Fig. 4A's legend.

Fig. 4C is a bar graph showing the stimulation of IP production in cells transfected with (1) 0.125 μg of pCMV6-SSTR3, and (2) 0.125 μg of plasmid encoding intact $G\alpha_q$. Experiments were performed as described in
15 Fig. 4A's legend.

Fig. 4D is a bar graph showing the stimulation of IP production in cells transfected with (1) 0.125 μg of plasmid encoding parathyroid hormone receptor (PTHR), and (2) 0.125 μg of plasmid encoding intact $G\alpha_q$. Experiments
20 were performed as described in Fig. 4A's legend.

Fig. 5A is a bar graph showing the effects of SST on AC activity in cells expressing a SSTR and $G\alpha_s/\alpha_{12}$. Cells were transfected with (1) 0.125 μg of plasmid encoding $G\alpha_s/\alpha_{12}$, and (2) 0.125 μg of pCMV6-SSTR1, pCMV6-
25 SSTR2, pCMV6-SSTR3, pCMV6-SSTR5, pCDNAI-SSTR4, or pCMV6. At 24 h after transfection, cells were stimulated with 1 μM SST and cAMP formation was measured.

Fig. 5B is a bar graph converted from Fig. 5A, showing the ratios of cAMP levels in the presence vs.
30 absence of SST.

Fig. 5C is a bar graph showing the effects of SST on AC activity in cells expressing a SSTR and $G\alpha_s/\alpha_{13}$. Cells were transfected with (1) 0.125 μg of plasmid encoding $G\alpha_s/\alpha_{13}$, and (2) 0.125 μg of pCMV6-SSTR1, pCMV6-

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SSTR2, pCMV6-SSTR3, pCMV6-SSTR5, pCDNAI-SSTR4, or pCMV6.
At 24 h after transfection, cells were stimulated with
1 μM SST and cAMP formation was measured.

5   **Fig. 5D** is a bar graph converted from Fig. 5C,
showing the ratios of cAMP levels in the presence vs.
absence of SST.

Detailed Description
Identification of the G Protein Gα Subunit that
Associates with a Given G-Linked Receptor

10   One feature of the present invention is a
comprehensive system wherein Gα subtype coupling can be
assigned for any given G-linked receptor. The following
examples are meant to illustrate, but not limit, the
methods of the present invention. Other suitable
15 modifications and adaptations of the conditions which are
obvious to those skilled in the art are within the scope
and spirit of the invention. For instance, genetically
engineered variants of G-linked receptors can be
substituted for the naturally occurring receptors.
20 Standard transfection techniques other than the
lipofection technique illustrated below, e.g., calcium
phosphate precipitation, biolistic transfer, DEAE-
Dextran, and viral-vector methods, can also be employed
in the invention.

25 **EXAMPLES**
Materials and Methods
Cells and Transfection
        COS cells were grown in Dulbecco's modified
Eagle's medium (DMEM) supplemented with 10% fetal calf
30 serum and antibiotics, as described previously (Ikezu et
al., J. Biol. Chem. 270: 29224-29228, 1995) Transient
transfection was performed by lipofection as previously
described (Ikezu et al., J. Biol. Chem. 270: 29224-29228,

1995).   In brief, $2x10^4$ cells were seeded onto a 24-well
plate and cultured in complete growth medium for 24 h.
The cells were subsequently transfected with 0.25 μg of
plasmid and 1 μl LipofectAMINE™ (GIBCO-BRL) for another

5   24 h in serum-free DMEM, and cultured in complete growth
medium for an additional 24 h.

<u>Measurement of AC activity</u>

        Intact-cell AC activity was assessed by measuring
cAMP formation as described previously (Ikezu et al., J.

10 Biol. Chem. 270: 29224-29228, 1995).   In brief, at 24 h
after transfection, cells were labeled with 6 μCi/ml of
[^{3}H]adenine (Du Pont-NEN) for 24 h, and then treated with
ligands of the G-linked receptor of interest (e.g.,
somatostatin-14 for a somatostatin receptor) and 1 mM

15 IBMX (3-isobutyl-1-methylxanthine) for 30 min.   The
resultant radioactive cAMP was separated on two-step
ion-exchange columns.   Specific accumulation of cAMP was
expressed as [cAMP/(ADP + ATP)] x 10^3, which represents
intact-cell AC activity.   Statistical analysis was

20 performed with Student's t test.

<u>Measurement of PI Turnover</u>

        PI (phosphatidyl inositol) turnover was assessed
by measuring IP (inositol phosphates) production.
$4x10^4$ cells were seeded onto a 24-well plate, cultured in

25 complete growth medium for 24 h, and transfected for 24 h
as described above.   The culture medium was replaced with
the labeling medium [inositol-free RPMI supplemented with
dialyzed fetal calf serum and 10 μCi/ml of
[^{3}H]myo-inositol (Du Pont-NEN)].   After incubation in the

30 labeling medium at 37°C for 12 h, the cells were washed
four times with inositol-free RPMI and treated with 1 μl
somatostatin (SST) in inositol-free RPMI at 37°C for
5 min.   After discarding the medium, the cells in 0.2 ml
fresh medium were lysed on the plate by 0.8 ml of

35 ice-cold 12.5% (final concentration: 10%) TCA, and the

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lysate was put on ice for 20 min before centrifugation. Supernatant of the lysate was mixed well with 1 ml of saturated diethyl ether to extract acid. After 5 repeated extractions, the collected sample was

5   neutralized with 1:100 dilution of concentrated ammonia, added to 4 ml water, and analyzed by a method described by Wu et al. (J. Biol. Chem. 267: 25798-25802, 1992) using Dowex column (AG 1-x8 Resin, 100-200 mesh, formate form, by BioRad).

10 Genes and Nucleic Acid Constructs

        cDNA expression constructs encoding somatostatin receptors (SSTR) types 1, 2, 3, and 5 have been previously described (Kubota et al., J. Clin. Invest. 93: 1321-1325, 1994; Kagimoto et al., Biochem. Biophys. Res.

15 Commun. 202: 1188-1195, 1994; Yamada et al., Mol. Endocrinol. 6: 2136-2142, 1992). These constructs (designated pCMV6-SSTR1, pCMV6-SSTR2, pCMV6-SSTR3, and pCMV6-SSTR5), all of which were derived from a pCMV6 vector (the SSTR1 and 2 constructs: pCMV6b; the SSTR3 and

20 5 constructs: pCMV6c), contain the SSTR coding sequences under the transcriptional control of the cytomegalovirus promoter. The SSTR4 expression construct (pcDNAI-SSTR4) was made by inserting the SSTR4 cDNA (Bito et al., J. Biol. Chem. 269: 12722-12730, 1994) in pBluescript

25 (Strategene) into pcDNAI (Invitrogen).

        The $G\alpha_s$ chimeras were constructed as follows. First, PCR was performed to add AflII and XbaI sites at the 3' end of the wild type $G\alpha_s$ cDNA using the following two primers:

30   ATCTGGAATAACAGATGGCTGC (SEQ ID NO:1) and

AAACTAGTCTAGACTAGCTCAAATTCTTAAGTGCATGCGCTGGATGATGTCA
        (SEQ ID NO:2).
The PCR product was digested with BglII and XbaI, and subcloned into pcDNAI-$G\alpha_s$ (i.e., the original plasmid

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containing the wild type Gαs cDNA) which had been
predigested with the same enzymes. The resultant
construct, designated Gα$_s$-AX, was sequenced to confirm
the presence of AflII and XbaI sites. Subsequently, the
5 construct was digested with AflII and XbaI, and ligated
with two synthetic oligonucleotides to add sequence
encoding the carboxyl-terminal five residues of a non-Gα$_s$
subunit. The oligonucleotides were:

   (1)   TTAAGAGATTGCGGCTTATTTTAAT (SEQ ID NO:3) and
10   CTAGATTAAAATAAGCCGCAATCTC (SEQ ID NO:4)
       (for Gα$_s$/α$_{i1}$);

   (2)   TTAAGAGAATGCGGCTTATTTTAAT (SEQ ID NO:5) and
        CTAGATTAAAATAAGCCGCATTCTC (SEQ ID NO:6)
       (for Gα$_s$/α$_{i3}$);

15 (3)   TTAAGAGGTTGCGGCTTGTACTAAT (SEQ ID NO:7) and
        CTAGATTAGTACAAGCCGCAACCTC (SEQ ID NO:8)
       (for Gα$_s$/α$_o$);

   (4)   TTAAGATACATCGGTTTGTGTTAAT (SEQ ID NO:9) and
        CTAGATTAACACAAACCGATGTATC (SEQ ID NO:10)
20   (for Gα$_s$/α$_z$);

   (5)   TTAAGAGAGTACAACCTCGTTTAAT (SEQ ID NO:11) and
        CTAGATTAAACGAGGTTGTACTCTC (SEQ ID NO:12)
       (for Gα$_s$/α$_q$);

   (6)   TTAAGAGATATCATGCTTCAATAAT (SEQ ID NO:13) and
25   CTAGATTATTGAAGCATGATATCTC (SEQ ID NO:14)
       (for Gα$_s$/α$_{12}$);

   (7)   TTAAGACAACTCATGCTTGAATAAT (SEQ ID NO:15) and
        CTAGATTATTCAAGCATGAGTTGTC (SEQ ID NO:16)
       (for Gα$_s$/α$_{13}$);

30 (8)   TTAAGAGAATTCAACTTAGTTTAAT (SEQ ID NO:17) and
        CTAGATTAAACTAAGTTGAATTCTC (SEQ ID NO:18)
       (for Gα$_s$/α$_{14}$); and

   (9)   TTAAGAGAGATCAATTTGTTGTAAT (SEQ ID NO:19) and
        CTAGATTACAACAAATTGATCTCTC (SEQ ID NO:20)
35   (for Gα$_s$/α$_{16}$).

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That the final products encoded the designed chimeric
sequences was verified by sequencing. Creation of the
AflII site in the $G\alpha_s$ cDNA did not change the encoded
sequence, and thus did not affect the sequence of the

5   $G\alpha_s/\alpha_x$ chimeras. Expression of the $G\alpha$ chimeras was
detected by a common $G\alpha$ antibody (UBI) in immunoblot
analysis. Rat parathyroid hormone (PTH) receptor cDNA
was provided by Dr. G. V. Segre. Receptor ligands
somatostatin-14 (SST-14, referred to as SST herein) and

10 PTH 1-34 were purchased from Sigma. SST-28 was obtained
from BACHEM.

Experimental Design and Results

Stimulation of $G\alpha_s$, but not any other $G\alpha$, results
in an increase in adenylyl cyclase (AC) activity. All

15 known types of AC can be stimulated by $G\alpha_s$. Thus, it is
possible to monitor the activity of $G\alpha_s$ by measuring the
rate of cAMP formation, a process catalyzed by AC.

It has been shown that the last 5 C-terminal
residues of at least some of $G\alpha$'s (e.g., $G\alpha_{i2}$ and $G\alpha_z$) is

20 the major determinant for the subunit's receptor-coupling
specificity (Conklin et al. Nature 363: 274-276, 1993 and
references therein; Voyno-Yasenetskaya et al., J. Biol.
Chem. 269: 4721-4724, 1994), and that a G-linked receptor
has to recognize these C-terminal residues before it can

25 exert its agonist-induced regulative effect. Thus, to
assess the $G\alpha$-coupling ability of any non-AC stimulating
(thus non-$G\alpha_s$-coupling) G-linked receptor, one can
utilize $G\alpha_s/\alpha_x$ ($G\alpha_x$: any type of $G\alpha$ except $G\alpha_s$) chimeras
wherein the last five C-terminal residues of the $G\alpha_s$

30 polypeptide are replaced with those of $G\alpha_x$. If a
receptor couples to $G\alpha_x$, it will, upon binding to its
ligand, recognize and activate the $G\alpha_s/\alpha_x$ chimera, thereby
resulting in $G\alpha_s$-mediated AC stimulation in the cell.

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$G\alpha_s/\alpha_x$ chimeras consisting of $G\alpha_s$ 1-389 (which lacks the original five C-terminal residues of $G\alpha_s$) and the five C-terminal residues of each known $G\alpha$ were constructed. The five C-terminal residues are identical

5   between $G\alpha_{i1}$ and $G\alpha_{i2}$, between $G\alpha_{o1}$ and $G\alpha_{o2}$, and between $G\alpha_q$ and $G\alpha_{11}$. Nine chimeras were constructed and designated $G\alpha_s/\alpha_{i1}$, $G\alpha_s/\alpha_{i3}$, $G\alpha_s/\alpha_o$, $G\alpha_s/\alpha_z$, $G\alpha_s/\alpha_q$, $G\alpha_s/\alpha_{12}$, $G\alpha_s/\alpha_{13}$, $G\alpha_s/\alpha_{14}$, and $G\alpha_s/\alpha_{16}$, respectively (Fig. 1). The residues 1-389 (SEQ ID NO:21) of $G\alpha_s$ are the

10   following:

      1 MGCLGNSKTE   DQRNEEKAQR   EANKKIEKQL   QKDKQVYRAT

     41 HRLLLLGAGE   SGKSTIVKQM   RILHVNGFNG   EGGEEDPQAA

     81 RSNSDGEKAT   KVQDIKNNLK   EAIETIVAAM   SNLVPPVELA

    121 NPENQFRVDY   ILSVMNVPDF   DFPPEFYEHA   KALWEDEGVR

15 161 ACYERSNEYQ   LIDCAQYFLD   KIDVIKQADY   VPSDQDLLRC

    201 RVLTSGIFET   KFQVDKVNFH   MFDVGGQRDQ   RRKWIQCFND

    241 VTAIIFVVAS   SSYNMVIRED   NQTNRLQEAL   NLFKSIWNNR

    281 WLRTISVILF   LNKQDLLAEK   VLAGKSKIED   YFPEFARYTT

    321 PEDATPEPGE   DPRVTRAKYF   IRDEFLRIST   ASGDGRHYCY

20 361 PHFTCAVDTE   NIRRVFNDCR   DIIQRMHLR

The experimental strategy was to transiently express a $G\alpha_s/\alpha_x$ cDNA along with a SSTR cDNA, and then to compare AC activities in the presence and absence of SST. If treatment with SST promotes cAMP formation only in

25   cells expressing the SSTR and a given $G\alpha_s/\alpha_x$, one can assume the linkage of the SSTR to that $G\alpha_s/\alpha_x$ and therefore to that $G\alpha_x$. The $G\alpha_s$ chimeras were each expressed as a 52-kDa protein at similar levels in COS cells, consistent with expected molecular weight.

30   The effect of SST on cAMP formation was first examined in cells transfected with a $G\alpha_s/\alpha_x$ construct alone (e.g., $G\alpha_s/\alpha_{i1}$, $G\alpha_s/\alpha_{i3}$, $G\alpha_s/\alpha_o$, $G\alpha_s/\alpha_z$, $G\alpha_s/\alpha_q$, $G\alpha_s/\alpha_{14}$, and $G\alpha_s/\alpha_{16}$). When an empty vector, rather than a SSTR cDNA construct, was transfected into these

chimera-expressing cells, SST had little effect on cAMP formation at up to

1 μM, as shown in Figs. 2B and 3B.

The next step was to confirm that the chimeras

5 were functional. For this purpose, $G\alpha_s/\alpha_x$ chimeras where $G\alpha_x$ is derived from the $G\alpha_i$ family ($G\alpha_i$, $G\alpha_o$, and $G\alpha_z$) were tested for their ability to transduce AC-stimulatory signal initiated by SST-bound SSTR3. SSTR3 has been shown to function as a G_i-coupled receptor and to

10 suppress AC activity in various cell types (Yasuda et al., J. Biol. Chem. 267: 20422-20428, 1992; Yamada et al., Mol. Endocrinol. 6: 2136-2142, 1992; Kaupmann et al., FEBS Lett. 331: 53-59, 1993; Law et al., Mol. Pharmacol. 45: 587-590, 1993 and Law et al., Mol.

15 Pharmacol. 45: 587-590, 1994; Patel et al., Biochem. Biophys. Res. Commun. 198: 605-612, 1994). Indeed, SST treatment resulted in inhibition of AC in COS cells transfected with a plasmid expressing SSTR3 (Fig. 2A). When cholera toxin, a potent stimulator of AC, was

20 employed to increase the basal AC level, the inhibition of AC by SST treatment was even more apparent (Fig. 2A).

In clear contrast to the decrease in AC in cells expressing SSTR3 alone, SST augmented AC activity in cells co-expressing SSTR3 and either $G\alpha_s/\alpha_{i1}$ or $G\alpha_s/\alpha_{i3}$

25 (Fig. 2C). Thus, the interaction between SSTR3 and $G\alpha_s/\alpha_i$ chimeras converted the effect of SSTR3 activation from AC inhibition to AC stimulation by switching the effector region of the Gα protein from that of $G\alpha_i$ to that of $G\alpha_s$, suggesting that the chimeras constructed herein were

30 operative.

Notably, in cells expressing SSTR3 and either $G\alpha_s/\alpha_o$ or $G\alpha_s/\alpha_z$, no augmentation of AC was observed (Figs. 2C and 2D). These data were consistent with multiple reports demonstrating the linkage of SSTR3

35 solely to $G\alpha_{i1}$, $G\alpha_{i2}$, and $G\alpha_{i3}$ of the $G\alpha_i$ family, which is

known to have at least 6 members. Given the fact that
the only $G\alpha$ proteins known to inhibit AC are members of
the $G\alpha_i$ family, which include the $G\alpha_i$'s (i.e., $G\alpha_{i1}$, $G\alpha_{i2}$,
$G\alpha_{i3}$), the $G\alpha_o$'s (i.e., $G\alpha_{o1}$ and $G\alpha_{o2}$), and $G\alpha_z$ (Wong et

5   al., Nature 351: 63-65, 1991), the present data suggest
that SSTR3 may inhibit cAMP formation exclusively through
the $G\alpha_i$'s.

     The next question was whether use of the remaining
chimeras can reveal any unknown linkage of SSTR3 to other

10 $G\alpha$'s, particularly those in the $G\alpha_q$ family. For this
purpose, an expression construct encoding SSTR3 and a
second expression construct encoding one of $G\alpha_s/\alpha_q$,
$G\alpha_s/\alpha_{14}$, and $G\alpha_s/\alpha_{16}$ were cotransfected into COS cells.
cAMP formation in these cells was measured. In cells

15 expressing SSTR3 and either $G\alpha_s/\alpha_{14}$ or $G\alpha_s/\alpha_{16}$, SST
treatment resulted in small, but statistically
significant increase in AC activity (Fig. 3A). Since SST
significantly reduced cAMP formation when SSTR3 was
transfected without $G\alpha_s/\alpha_{14}$ or $G\alpha_s/\alpha_{16}$ (Fig. 2A), it is

20 conceivable that the net stimulation of AC by SSTR3
through these two chimeras may have been considerably
larger than what was observed. In contrast, in cells co-
expressing SSTR3 and $G\alpha_s/\alpha_q$, no stimulation of AC was
observed under the same conditions.

25   Figs. 3C and 3D show results of the experiments
wherein the linkage of SSTR3 to chimeras derived from the
$G\alpha_i$ and $G\alpha_q$ families were examined in parallel. Again,
the results demonstrated that SSTR3 may link to $G\alpha_{14}$ and
$G\alpha_{16}$, in addition to the $G\alpha i$'s, but not to any other

30 members of the $G\alpha_i$ and $G\alpha_q$ families .

     The putative linkage of SSTR3 to $G\alpha_{14}$ and $G\alpha_{16}$ was
confirmed by use of the full length $G\alpha_{14}$ and $G\alpha_{16}$
molecules. It is known that, when linked to an
appropriately liganded receptor, $G\alpha_q$, $G\alpha_{11}$, $G\alpha_{14}$ and $G\alpha_{16}$

35 are all able to stimulate phospholipase C (PLC). Thus, a

functional linkage between SSTR3 and any of these four
molecules can be demonstrated by SST-initiated PI
turnover in cells co-expressing SSTR3 and the Gα
molecule. As shown in Fig. 4A, SST stimulated IP
5 production when both $G\alpha_{16}$ and SSTR3 were expressed in COS
cells. No PI turnover was observed when either molecule
was expressed alone. In addition, consistent with the
resistance of $G\alpha_{16}$ to pertussis toxin (PTX), PTX failed to
affect this stimulation (Fig. 4A). Similarly, when SSTR3
10 was co-expressed with $G\alpha_{14}$, SST led to a statistically
significant, though lower, increase of IP production
(Fig. 4B). Again, no PI turnover was observed when
either molecule was expressed alone. In contrast, when
SSTR3 was co-expressed with $G\alpha_q$, SST had no effect on IP
15 production (Fig. 4C). These data demonstrated that a
linkage between a given G-linked receptor and a given
$G\alpha_s/\alpha_x$ chimera is predictive of a linkage between the
receptor and that particular $G\alpha_x$.

Under the same conditions, transfection of cDNA
20 encoding the parathyroid hormone receptor (PTHR) with or
without cDNA for $G\alpha_q$ resulted in significant stimulation
of IP production in response to maximal PTH stimulation
(Fig. 4D). Transfection of $G\alpha_q$ augmented the ability of
PTHR to activate PLC. These data are consistent with the
25 observations that (i) PTHR causes PI turnover in a
PTX-insensitive manner (Iida-Klein et al., J. Biol. Chem.
270: 8458-8465, 1995), and (ii) COS cells endogenously
express $G\alpha_q$ and $G\alpha_{11}$ (Wu et al., J. Biol. Chem. 267:
25798-25802, 1992). Therefore, SSTR3-mediated $G\alpha_{16}$
30 stimulation, which even exceeded PTHR-mediated $G\alpha_q$
stimulation, is specific and significant.

Interestingly, despite that SSTR3 coupled to
$G\alpha_s/\alpha_{14}$ and $G\alpha_s/\alpha_{16}$ with similar efficiency (Figs. 3C and
3D), it coupled to intact $G\alpha_{16}$ far more efficient than to
35 intact $G\alpha_{14}$ (Figs. 4A and 4B). This finding suggests that

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the extreme C-terminal region of a Gα be the major but
not sole determinant for the Gα's full interaction with
its linked receptor. Other regions of the Gα polypeptide
may also involve, as shown by a number of other studies.

5   In summary, inability of a G-linked receptor to
couple to a given $G\alpha_s/\alpha_x$ indicates the inability of the
receptor to recognize the C terminus of that particular
$G\alpha_x$, and therefore rules out the coupling between the
receptor and the intact $G\alpha_x$. In this context, the

10 present study suggests for the first time that SSTR3 may
not couple to $G\alpha_o$, $G\alpha_z$, $G\alpha_q$, $G\alpha_{11}$, $G\alpha_{12}$, or $G\alpha_{13}$ (see below
for $G\alpha_{12}$ and $G\alpha_{13}$). On the other hand, ability of a G-
linked receptor to couple to a given $G\alpha_s/\alpha_x$ indicates that
the receptor is capable of coupling to that particular

15 $G\alpha_x$.

           The present chimeric system is extremely useful in
identifying potential receptor-Gα linkage, especially for
Gα's which have less established signal-transducing
effectors and which therefore are less amenable to

20 assaying. In this regard, the present system can be
employed to identify $G\alpha_{12}$- or $G\alpha_{13}$-coupled receptors.
Although $G\alpha_{12}$ and $G\alpha_{13}$ have been implicated in pivotal
cellular functions (Voyno-Yasenetskaya et al., Oncogene
9: 2559-2565, 1994 and Voyno-Yasenetskaya et al., J.

25 Biol. Chem. 269: 4721-4724, 1994), receptors to which
they couple remain elusive.

           To investigate whether $G\alpha_{12}$ and $G\alpha_{13}$ couple to any
of the 5 known subtypes of SSTR's, SST-induced cAMP
formation was measured in cells co-expressing a given

30 SSTR and either $G\alpha_s/G\alpha_{12}$ or $G\alpha_s/G\alpha_{13}$. Figs. 5A-5D shows
that $G\alpha_s/G\alpha_{12}$ was activated by SSTR2, 4, and 5 in the
order of SSTR5 >> SSTR2 ≈ SSTR4, while $G\alpha_s/G\alpha_{13}$ was
activated almost exclusively by SSTR5. Notably, the
stimulation of $G\alpha_s/G\alpha_{12}$ and $G\alpha_s/G\alpha_{13}$ by liganded SSTR5

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yielded a more than 5 fold increase in the cAMP level
(Figs. 5B and 5D).

**Table I. Dose effects of SST (SST-14) and SST-28 on cAMP
formation in chimera-expressing cells**

Dose(M)	SSTR5 transfected cells		SSTR2 transfected cells	
	SST	SST-28	SST	SST-28
10^{-10}	104.1±4.5	98.9±2.1		
10^{-9}	90.4±4.3	95.2±4.1		
10^{-8}	102.5±4.2	98.5±8.6	94.1±9.0	107.0±3.3*
10^{-7}	282.3±2.1	359.8±3.6*	129.5±1.3	112.8±9.9*
10^{-6}	418.4±4.1	463.5±2.8*	202.5±15.3	187.7±2.9

After transfection of $G\alpha_s/\alpha_{12}$ chimera and SSTR2 or SSTR5
cDNA, cells were stimulated with various concentrations
of SST-14 or SST-28, and cAMP formation was measured.
The results are indicated as percentage relative to cAMP
formation at 10^{-11} M SST or SST-28, which was similar to
basal formation shown in Fig. 5.   Data are presented as
means ± S.E. of quadruplicated experiments.

        Table I shows that, in the presence of $G\alpha_s/\alpha_{12}$,
the stimulation of cAMP formation by SSTR5 and SSTR2 is
SST-dosage-dependent and biphasic.   At low SST
concentrations, cAMP formation was slightly but
reproducibly inhibited, whereas at higher concentrations,
cAMP formation was strongly stimulated.   It is
conceivable that the former effect may be mediated by
SSTR2/5's coupling to the endogenous $G\alpha_i$, and the latter

by SSTR2/5's coupling to $G\alpha_s/\alpha_{12}$. Several lines of
evidence showed that the observed AC stimulation was not
mediated by the $G\beta\gamma$ subunit released from the endogenous
G_i.

5   Table I also indicates that SST-28 had a more
potent effect on the function of SSTR5 than the naturally
occurring SST (i.e., SST-14), regarding both of their
inhibitory and stimulatory effects. These findings are
consistent with the well known fact that SSTR5 has a
10 higher affinity for SST-28 than for SST-14, while other
SSTR's have a lower affinity for SST-28.
        In addition to use in identifying a potential
linkage between a given G-linked receptor and a given $G\alpha$
having less established signal-transducing effectors, the
15 present system can also be used to investigate proteins
with a G-linked-receptor-like structure (e.g., with
multiple transmembrane domains) but having unknown
functions. The system can also be used to investigate
proteins which have only a single-transmembrane domain
20 but which are suspected of being a G-linked receptor.
Examples of these proteins are insulin-like growth factor
II receptor (Murayama et al., J. Biol. Chem. 265: 17456-
17462, 1990), amyloid precursor protein (APP) (Okamoto et
al., FEBS Lett. 334: 143-148, 1995), and sperm β-
25 1,4-galactosyltransferase (Gong et al., Science, 269:
1718-1721, 1995). There are many other single-spanning
proteins with a possible G-coupling ability, examples of
which include epidermal growth factor receptor (Sun et
al., Proc. Natl. Acad. Sci. U.S.A., 92: 2229-2233, 1995),
30 insulin receptor (Luttrell et al., J. Biol. Chem., 265:
16873-16879, 1990; Okamoto et al., FEBS Lett., 334: 143-
148, 1994), and insulin-like growth factor I (IGF-I)
receptor (Nishimoto et al., Biochem. Biophys. Res.
Commun. 148: 407-412, 1987; Luttrell et al., J. Biol.
35 Chem. 270: 16495-16498, 1995). The present system can be

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employed to examine the Gα-coupling potential of these candidates as well.

Identification of Compounds Capable of Modulating the Interaction between a G-linked Receptor and Its Coupled
5 ## Non-Gα$_s$ Gα Subunit

One aspect of the present invention is a method of identifying a compound that can modulate the interaction between a G-linked receptor and the Gα subunit of a non-G$_s$ G protein known to couple to the receptor. In the
10 claimed method, two samples of cells are provided, both of which express (a) the receptor of interest, and (b) a chimeric polypeptide containing amino acid residues 1-389 of Gα$_s$ (SEQ ID NO:21) followed by the C-terminal 5 amino acid residues of the non-Gα$_s$ Gα subunit known to couple
15 to the receptor. A ligand of the G-linked receptor is administered to both cell samples. Prior to, subsequent to, or at the same time as the ligand administration, the second cell is contacted with a candidate compound. Then the activity of adenylyl cyclase in each cell sample is
20 determined and compared as described above. A statistically significant (i.e., $p<0.05$ in Student's t test) change of the AC activity in the second cell sample as compared to the first cell sample indicates that the compound may be capable of modulating the interaction
25 between the G-linked receptor and the coupling Gα subunit. For example, a statistically significant decrease of the AC activity in the compound-contacted cells will suggest that the compound may block the interaction. The efficacy of the compound can be
30 confirmed by a second assay using the full length Gα subunit instead of the chimera.

Cell lines that can be used in connection with this method include those of mammalian origin, such as COS cells and HEK 293 cells (American Type Culture

Collection). Maintenance and transfection of such cells
can be performed using well known methods. Proteins (a)
and (b) (see above) can be introduced into the target
cells via transfection of nucleic acid constructs

5   encoding them. Techniques for making nucleic acid
constructs are well known in the art (see, e.g., Sambrook
et al., Molecular Cloning, a Laboratory Manual, Cold
Spring Harbor Laboratory, Cold Spring Harbor, New York,
1989); examples of such techniques have been illustrated

10 above.

        G-linked receptors of interest include, but are
not limited to, those described in U.S. Patent
No.5,559,209, herein incorporated by reference (e.g.,
insulin-like growth factor II receptor, muscarinic

15 acetylcholine receptor, α_2-adrenergic receptor, adenosine
receptor, thrombin receptor, transforming growth factor β
receptor, T cell receptor, PTH/PTHrP receptor, calcitonin
receptor, endothelin receptor, angiotensin receptor,
platelet activating factor receptor, thromboxane A_2

20 receptor, any of the somatostatin receptors, D_2-dopamine
receptor, γ-butyric acid receptor), and amyloid protein
precursor (APP).

        Nucleic acid constructs that permit expression of
SSTR1, 3, and 5 in COS cells have been described above.

25 APP has at least 10 isoforms, one of which (APP_{695}) is
preferentially expressed in neuronal tissue (Sandbrink et
al., J. Biol. Chem. 269: 1510, 1994). The construction
of a baculovirus construct containing the APP_{695} cDNA has
been described (Nishimoto et al., Nature 362: 75-79,

30 1993). Similar cloning techniques can be employed to
create APP_{695} mammalian expression constructs based on
mammalian expression vectors such as pCDNAI and pCMV6.

        Constitutively active variants of the G-linked
receptors can also be used in the present screening

35 method, eliminating the need for their ligands. For

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instance, three constitutively active APP_{695} mutants, designated Ile-APP, Phe-APP, and Gly-APP, have been identified in familial Alzheimer's Disease patients (Yamatsuji et al., Science 272: 1349-1352, 1996; and
5 references therein). These three mutants have mis-sense mutations in which Val^{642} in the transmembrane domain of APP_{695} is replaced by Ile, Phe, or Gly, respectively.

Alteration of the Signal-Transducing Output of a G-Linked Receptor

10   The chimeras of the invention can alternatively be used in a method of altering the signal-transducing output of a G-linked receptor. Abnormalities of G-linked receptor functions have been implicated in many significant diseases such as familial Alzheimer's disease
15 (Nishimoto et al., Nature 362: 75-79, 1993; Yamatsuji et al., Science 272: 1349-1352, 1996; Okamoto et al., The EMBO J. 15: 3769-3777, 1996; Ikezu et al., The EMBO J. 15: 2468-2475, 1996; and references therein), atherosclerosis, retinitis pigmentosa, malignant thyroid
20 tumor, precocious puberty, and familial hypocalcineuric hypercalcemia (Clapham, Cell 75: 1237-1239, 1993; Lefkowitz, Nature 365: 603-604, 1993).

     Amyloid protein precursor (APP), a G-linked cell surface receptor, has been shown to be mutated and
25 constitutively active in at least some forms of familial Alzheimer's Disease (Okamoto et al., The EMBO J. 15: 3769-3777, 1996; and references therein). APP is known to couple to G_o, the activation of which inhibits adenylyl cyclase (Okamoto et al., The EMBO J. 15: 3769-
30 3777, 1996 and references therein). Thus, changing the effector function of the G protein with which APP associates from inhibitory to stimulatory or neutral with regard to AC activity is expected to alleviate the symptoms of familial Alzheimer's Disease, and by

extension, any form of Alzheimer's Disease characterized
by constitutive or other inappropriately activation of
the receptor or its G protein.

The present invention provides a method for
5 augmenting adenylyl cyclase activity in brain neurons of
a mammal, and preferably, of a familial Alzheimer's
patient. In this method, a $G\alpha_s$ subunit in which the C-
terminal 5 aa residues are replaced with those of $G\alpha_o$ is
introduced into the brain neurons of the mammal. This
10 chimeric $G\alpha$ molecule will compete with the endogenous $G\alpha_o$
for the binding of APP, and upon binding to APP, will
transduce stimulatory signals to adenylyl cyclase,
thereby counteracting the inhibitory signals transduced
by native G_o. This chimeric molecule can be introduced
15 into the target cell by overexpressing within the target
cell a nucleic acid construct comprising a promoter
sequence operably linked to a sequence encoding the
protein. The nucleic acid construct is typically derived
from a non-replicating linear or circular DNA or RNA
20 vector, or from an autonomously replicating plasmid or
viral vector; or the construct is integrated into the
host genome. These nucleic acid constructs can be made
with methods well known in the art (see, e.g., Sambrook
et al., Molecular Cloning, A Laboratory Manual, Cold
25 Spring Harbor Press, Cold Spring Harbor, New York, 1989).
Any vector that can transfect a brain neuron may be used
in the method of the invention. A preferred vector is a
herpes simplex viral (HSV) vector or an appropriately
modified version of this vector.
30   A therapeutic composition containing this vector
may be used alone or in a mixture, or in chemical
combination, with one or more materials, including other
proteins or recombinant vectors that increase the
biological stability of the recombinant vectors, or with
35 materials that increase the therapeutic composition's

ability to penetrate the target tissue selectively. The
therapeutic compositions of the invention is typically
administered in a pharmaceutically acceptable carrier
(e.g., physiological saline), which is selected on the
5 basis of the mode and route of administration, and
standard pharmaceutical practice. Suitable
pharmaceutical carriers, as well as pharmaceutical
necessities for use in pharmaceutical formulations, are
described in *Remington's Pharmaceutical Sciences*, a
10 standard reference text in this field, and in the USP/NF.
        The therapeutic compositions of the invention can
be administered in dosages determined to be appropriate
by one skilled in the art. It is expected that the
dosages will vary, depending upon the pharmacokinetic and
15 pharmacodynamic characteristics of the particular agent,
and its mode and route of administration, as well as the
age, weight, and health of the recipient; the nature and
extent of the disease; the frequency and duration of the
treatment; the type of, if any, concurrent therapy; and
20 the desired effect.
        The therapeutic compositions may be administered
to a patient by any appropriate mode, e.g., via applying
drops or spray onto the nasal mucosa, or via injection
into the nasal mucosa, as determined by one skilled in
25 the art. Alternatively, it may be desired to administer
the treatment surgically to the target tissue. The
treatments of the invention may be repeated as needed, as
determined by one skilled in the art.

Inhibition of Tumor Growth

30   By using the chimeric Gα system of the present
invention, $G\alpha_{12}$ and $G\alpha_{13}$ have been shown to couple to
SSTR5 (see above). These two Gα's, which have been
implicated as transducing apoptosis-generating and cell-
proliferation-inhibiting signals, are ubiquitously

expressed in human cells. Thus, the invention includes a
method of inhibiting tumor growth by expressing an
exogenously introduced SSTR5 protein, e.g., a recombinant
protein comprising (a) SSTR5, or (b) a biologically

5   active fragment thereof, in a tumor cell. Recombinant
$G\alpha_{12}$ or $G\alpha_{13}$ polypeptides can also be introduced into the
target cell. Upon administration of SST or its
biologically active analogue, the recombinant SSTR5
present on the cell surface will be stimulated and will

10 thereby inhibit growth of the tumor cell via endogenous
or recombinant $G\alpha_{12}$ and $G\alpha_{13}$.

This aspect of the invention is useful in cancer
treatments using SST-related drugs (i.e., SST or SST
analogues). Such treatments frequently lead to loss of

15 SSTR's naturally expressed on cancer cells, thereby
desensitizing the cells to the SST-related drugs.
Introduction of recombinant SSTR5 into the cancer cells
solves this problem, at least temporarily; further
transfetions may be necessary to maintain the effect, if

20 the recombinant SSTR5 is lost as well. All cancers,
including highly malignant ones such as pancreatic cancer
and small cell lung cancer, can be treated by the present
method. The recombinant SSTR5 protein can be introduced
into the cancer cells by overexpressing within the cells

25 a nucleic acid construct comprising a mammalian promoter
sequence operably linked to a sequence encoding the
protein. Preferably, the construct primarily targets
fast-proliferating cells, and can, for example, be
derived from retroviral, adenoviral, adeno-associated-

30 viral, or herpes simplex viral vectors, or any
appropriately modified versions of these vectors.
Retroviral vectors are particularly appropriate, as they
selectively integrate into the genome of replicating
cells, such as tumor cells. Methods for constructing

35 expression vectors are well known in the art (see, e.g.,

Sambrook et al., Molecular Cloning, A Laboratory Manual,
Cold Spring Harbor Laboratory, Cold Spring Harbor, New
York, 1989). The administration of SST, or its analogue,
and a therapeutic composition comprising the SSTR5

5   construct can be conducted using guidelines described in
the previous section.

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SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: The General Hospital Corporation

(ii) TITLE OF THE INVENTION: G-LINKED RECEPTORS

(iii) NUMBER OF SEQUENCES: 31

(iv) CORRESPONDENCE ADDRESS:
   (A) ADDRESSEE: Fish & Richardson P.C.
   (B) STREET: 225 Franklin Street
   (C) CITY: Boston
   (D) STATE: MA
   (E) COUNTRY: USA
   (F) ZIP: 02110-2804

(v) COMPUTER READABLE FORM:
   (A) MEDIUM TYPE: Diskette
   (B) COMPUTER: IBM Compatible
   (C) OPERATING SYSTEM: DOS
   (D) SOFTWARE: FastSEQ Version 2.0

(vi) CURRENT APPLICATION DATA:
   (A) APPLICATION NUMBER:
   (B) FILING DATE:
   (C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
   (A) APPLICATION NUMBER: 60/028,340
   (B) FILING DATE: 11-OCT-1996

(viii) ATTORNEY/AGENT INFORMATION:
   (A) NAME: Fraser, Janis K
   (B) REGISTRATION NUMBER: 34,819
   (C) REFERENCE/DOCKET NUMBER: 08472/706WO1

(ix) TELECOMMUNICATION INFORMATION:
   (A) TELEPHONE: 617-542-5070
   (B) TELEFAX: 617-542-8906
   (C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 22 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATCTGGAATA ACAGATGGCT GC   22

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 52 base pairs
   (B) TYPE: nucleic acid

- 30 -

            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: DNA

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

AAACTAGTCT AGACTAGCTC AAATTCTTAA GTGCATGCGC TGGATGATGT CA   52

            (2) INFORMATION FOR SEQ ID NO:3:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 25 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: DNA

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TTAAGAGATT GCGGCTTATT TTAAT   25

            (2) INFORMATION FOR SEQ ID NO:4:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 25 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: DNA

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CTAGATTAAA ATAAGCCGCA ATCTC   25

            (2) INFORMATION FOR SEQ ID NO:5:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 25 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: DNA

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TTAAGAGAAT GCGGCTTATT TTAAT   25

            (2) INFORMATION FOR SEQ ID NO:6:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 25 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CTAGATTAAA ATAAGCCGCA TTCTC   25

            (2) INFORMATION FOR SEQ ID NO:7:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 25 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TTAAGAGGTT GCGGCTTGTA CTAAT   25

            (2) INFORMATION FOR SEQ ID NO:8:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 25 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CTAGATTAGT ACAAGCCGCA ACCTC   25

            (2) INFORMATION FOR SEQ ID NO:9:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 25 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TTAAGATACA TCGGTTTGTG TTAAT   25

            (2) INFORMATION FOR SEQ ID NO:10:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 25 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

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CTAGATTAAC ACAAACCGAT GTATC   25

        (2) INFORMATION FOR SEQ ID NO:11:

      (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 25 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TTAAGAGAGT ACAACCTCGT TTAAT   25

        (2) INFORMATION FOR SEQ ID NO:12:

      (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 25 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CTAGATTAAA CGAGGTTGTA CTCTC   25

        (2) INFORMATION FOR SEQ ID NO:13:

      (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 25 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TTAAGAGATA TCATGCTTCA ATAAT   25

        (2) INFORMATION FOR SEQ ID NO:14:

      (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 25 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CTAGATTATT GAAGCATGAT ATCTC   25

          (2) INFORMATION FOR SEQ ID NO:15:

     (i) SEQUENCE CHARACTERISTICS:
         (A) LENGTH: 25 base pairs
         (B) TYPE: nucleic acid
         (C) STRANDEDNESS: single
         (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TTAAGACAAC TCATGCTTGA ATAAT   25

          (2) INFORMATION FOR SEQ ID NO:16:

     (i) SEQUENCE CHARACTERISTICS:
         (A) LENGTH: 25 base pairs
         (B) TYPE: nucleic acid
         (C) STRANDEDNESS: single
         (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CTAGATTATT CAAGCATGAG TTGTC   25

          (2) INFORMATION FOR SEQ ID NO:17:

     (i) SEQUENCE CHARACTERISTICS:
         (A) LENGTH: 25 base pairs
         (B) TYPE: nucleic acid
         (C) STRANDEDNESS: single
         (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

TTAAGAGAAT TCAACTTAGT TTAAT   25

          (2) INFORMATION FOR SEQ ID NO:18:

     (i) SEQUENCE CHARACTERISTICS:
         (A) LENGTH: 25 base pairs
         (B) TYPE: nucleic acid
         (C) STRANDEDNESS: single
         (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CTAGATTAAA CTAAGTTGAA TTCTC   25

          (2) INFORMATION FOR SEQ ID NO:19:

      (i) SEQUENCE CHARACTERISTICS:

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```
        (A) LENGTH: 25 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TTAAGAGAGA TCAATTTGTT GTAAT                                    25


            (2) INFORMATION FOR SEQ ID NO:20:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 25 base pairs
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CTAGATTACA ACAAATTGAT CTCTC                                    25


            (2) INFORMATION FOR SEQ ID NO:21:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 389 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS: N/A
            (D) TOPOLOGY: N/A

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Met Gly Cys Leu Gly Asn Ser Lys Thr Glu Asp Gln Arg Asn Glu Glu
1               5                   10                  15
Lys Ala Gln Arg Glu Ala Asn Lys Lys Ile Glu Lys Gln Leu Gln Lys
            20                  25                  30
Asp Lys Gln Val Tyr Arg Ala Thr His Arg Leu Leu Leu Leu Gly Ala
        35                  40                  45
Gly Glu Ser Gly Lys Ser Thr Ile Val Lys Gln Met Arg Ile Leu His
    50                  55                  60
Val Asn Gly Phe Asn Gly Glu Gly Gly Glu Glu Asp Pro Gln Ala Ala
65                  70                  75                  80
Arg Ser Asn Ser Asp Gly Glu Lys Ala Thr Lys Val Gln Asp Ile Lys
                85                  90                  95
Asn Asn Leu Lys Glu Ala Ile Glu Thr Ile Val Ala Ala Met Ser Asn
            100                 105                 110
Leu Val Pro Pro Val Glu Leu Ala Asn Pro Glu Asn Gln Phe Arg Val
            115                 120                 125
Asp Tyr Ile Leu Ser Val Met Asn Val Pro Asp Phe Asp Phe Pro Pro
        130                 135                 140
Glu Phe Tyr Glu His Ala Lys Ala Leu Trp Glu Asp Glu Gly Val Arg
145                 150                 155                 160
Ala Cys Tyr Glu Arg Ser Asn Glu Tyr Gln Leu Ile Asp Cys Ala Gln
                165                 170                 175
Tyr Phe Leu Asp Lys Ile Asp Val Ile Lys Gln Ala Asp Tyr Val Pro
            180                 185                 190
Ser Asp Gln Asp Leu Leu Arg Cys Arg Val Leu Thr Ser Gly Ile Phe
            195                 200                 205
```

```
Glu Thr Lys Phe Gln Val Asp Lys Val Asn Phe His Met Phe Asp Val
    210             215             220
Gly Gly Gln Arg Asp Gln Arg Arg Lys Trp Ile Gln Cys Phe Asn Asp
225             230             235                         240
Val Thr Ala Ile Ile Phe Val Val Ala Ser Ser Tyr Asn Met Val
            245             250             255
Ile Arg Glu Asp Asn Gln Thr Asn Arg Leu Gln Glu Ala Leu Asn Leu
        260             265             270
Phe Lys Ser Ile Trp Asn Asn Arg Trp Leu Arg Thr Ile Ser Val Ile
        275             280             285
Leu Phe Leu Asn Lys Gln Asp Leu Leu Ala Glu Lys Val Leu Ala Gly
    290             295             300
Lys Ser Lys Ile Glu Asp Tyr Phe Pro Glu Phe Ala Arg Tyr Thr Thr
305             310             315                         320
Pro Glu Asp Ala Thr Pro Glu Pro Gly Glu Asp Pro Arg Val Thr Arg
            325             330             335
Ala Lys Tyr Phe Ile Arg Asp Glu Phe Leu Arg Ile Ser Thr Ala Ser
            340             345             350
Gly Asp Gly Arg His Tyr Cys Tyr Pro His Phe Thr Cys Ala Val Asp
        355             360             365
Thr Glu Asn Ile Arg Arg Val Phe Asn Asp Cys Arg Asp Ile Ile Gln
370             375             380
Arg Met His Leu Arg
385
```

        (2) INFORMATION FOR SEQ ID NO:22:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 5 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS: N/A
            (D) TOPOLOGY: N/A

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```
Asp Cys Gly Leu Phe
1               5
```

        (2) INFORMATION FOR SEQ ID NO:23:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 5 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS: N/A
            (D) TOPOLOGY: N/A

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```
Glu Cys Gly Leu Tyr
1               5
```

        (2) INFORMATION FOR SEQ ID NO:24:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 5 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS: N/A
            (D) TOPOLOGY: N/A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Gly Cys Gly Leu Tyr
1   5

        (2) INFORMATION FOR SEQ ID NO:25:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 5 amino acids
       (B) TYPE: amino acid
       (C) STRANDEDNESS: N/A
       (D) TOPOLOGY: N/A

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Tyr Ile Gly Leu Cys
1   5

        (2) INFORMATION FOR SEQ ID NO:26:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 5 amino acids
       (B) TYPE: amino acid
       (C) STRANDEDNESS: N/A
       (D) TOPOLOGY: N/A

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Glu Tyr Asn Leu Val
1   5

        (2) INFORMATION FOR SEQ ID NO:27:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 5 amino acids
       (B) TYPE: amino acid
       (C) STRANDEDNESS: N/A
       (D) TOPOLOGY: N/A

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Asp Ile Met Leu Gln
1   5

        (2) INFORMATION FOR SEQ ID NO:28:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 5 amino acids
       (B) TYPE: amino acid
       (C) STRANDEDNESS: N/A
       (D) TOPOLOGY: N/A

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Gln Leu Met Leu Glu
1   5

(2) INFORMATION FOR SEQ ID NO:29:

    (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 5 amino acids
      (B) TYPE: amino acid
      (C) STRANDEDNESS: N/A
      (D) TOPOLOGY: N/A

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Glu Phe Asn Leu Val
1   5

(2) INFORMATION FOR SEQ ID NO:30:

    (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 5 amino acids
      (B) TYPE: amino acid
      (C) STRANDEDNESS: N/A
      (D) TOPOLOGY: N/A

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Glu Ile Asn Leu Leu
1   5

(2) INFORMATION FOR SEQ ID NO:31:

    (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 5 amino acids
      (B) TYPE: amino acid
      (C) STRANDEDNESS: N/A
      (D) TOPOLOGY: N/A

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Gln Tyr Glu Leu Leu
1   5

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We claim:

      1.   A method of determining whether a given G-linked receptor associates with the Gα subunit of a non-G_s G protein, said method comprising:

5   (1) providing a first cell and a second cell of the same cell type, each of which expresses

               (a) said receptor, and

               (b) a chimeric polypeptide comprising

                    (i) a first amino acid sequence

10 corresponding to residues 236-356 of SEQ ID NO:21, and

                    (ii) a second amino acid sequence 4-30 amino acids in length and corresponding to a segment of said Gα subunit, which segment ends at and includes the C-terminal residue of said Gα subunit;

15   (2) contacting said second cell with a ligand of said receptor; and

          (3) comparing the activity levels of adenylyl cyclase in said first and second cells, wherein a higher level in said second cell than in said first cell

20 indicates that said receptor associates with said Gα subunit.

      2.   The method of claim 1, wherein said first amino acid sequence comprises amino acid residues 1-389 of SEQ ID NO:21, and said second amino acid sequence

25 comprises the C-terminal 5 amino acid residues of said Gα subunit.

      3.   The method of claim 2, wherein said C-terminal 5 amino acid residues are selected from the group consisting of SEQ ID NOs: 22-30.

4.   A method of identifying a compound that can
modulate the interaction between a given G-linked
receptor and the Gα subunit of a non-G$_s$ G protein known
to couple to said receptor, said method comprising:

5   (1) providing a first cell and a second cell of
the same cell type, each of which expresses
                 (a) said receptor, and
                 (b) a chimeric polypeptide comprising
                      (i) a first amino acid sequence
10 corresponding to residues 236-356 of SEQ ID NO:21, and
                      (ii) a second amino acid sequence 4-30
amino acids in length and corresponding to a segment of
said Gα subunit, which segment ends at and includes the
C-terminal residue of said Gα subunit;

15   (2) contacting said first cell with a ligand of
said receptor;
           (3) contacting said second cell with said ligand
in the presence of a candidate compound; and
           (4) comparing the activity levels of adenylyl
20 cyclase in said first and second cells, wherein a higher
or lower level in said second cell than in said first
cell indicates that the compound modulates said
interaction.

5.   The method of claim 4, wherein said first
25 amino acid sequence comprises amino acid residues 1-389
of SEQ ID NO:21, and said second amino acid sequence
comprises the C-terminal 5 amino acid residues of said Gα
subunit.

6.   The method of claim 4, wherein said receptor
30 is somatostatin receptor type 1 and said Gα subunit is
Gα_i or Gα_z.

7.   The method of claim 4, wherein said receptor is somatostatin receptor type 3 and said $G\alpha$ subunit is $G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$, $G\alpha_{14}$, or $G\alpha_{16}$.

8.   The method of claim 4, wherein said receptor
5   is somatostatin receptor type 5 and said $G\alpha$ subunit is $G\alpha_{12}$ or $G\alpha_{13}$.

9.   The method of claim 4, wherein said receptor is insulin-like growth factor II receptor, muscarinic acetylcholine receptor, D_2-dopamine receptor, α_2-
10 adrenergic receptor, adenosine receptor, thrombin receptor, or transforming growth factor β receptor; and said $G\alpha$ subunit is $G\alpha_{i1}$, $G\alpha_{i2}$, or $G\alpha_{i3}$.

10.   The method of claim 4, wherein said receptor is amyloid protein precursor (APP), transforming growth
15 factor-β receptor, γ-butyric acid receptor, muscarinic acetylcholine receptor, adenosine receptor, thrombin receptor, or α_2-adrenergic receptor; and said $G\alpha$ subunit is $G\alpha_o$.

11.   The method of claim 4, wherein said receptor
20 is the T cell receptor, PTH/PTHrP receptor, calcitonin receptor, endothelin receptor, angiotensin receptor, platelet activating factor receptor, or thromboxane A_2 receptor; and said $G\alpha$ subunit is $G\alpha_q$.

12.   A method of identifying a compound that can
25 modulate the interaction between $G\alpha_o$ and a constitutively active mutant of APP, said method comprising:
        (1) providing a first cell and a second cell of the same cell type, each of which expresses
              (a) said mutant, and
30   (b) a chimeric polypeptide comprising

(i) a first amino acid sequence
corresponding to residues 236-356 of SEQ ID NO:21, and
(ii) a second amino acid sequence 4-30
amino acids in length and corresponding to a segment of
5 $G\alpha_o$, which segment ends at and includes the C-terminal
residue of said $G\alpha_o$;
(2) contacting said second cell with a candidate
compound; and
(3) comparing the activity levels of adenylyl
10 cyclase in said first and second cells, wherein a higher
or lower level in said second cell than in said first
cell indicates that the compound modulates said
interaction.

13.   The method of claim 12, wherein said first
15 amino acid sequence comprises amino acid residues 1-389
of SEQ ID NO:21, and said second amino acid sequence
comprises the C-terminal 5 amino acid residues of $G\alpha_o$.

14.   The method of claim 12, wherein said mutant
is Ile-APP, Phe-APP, or Gly-APP.

20   15.   A method of altering the signal-transducing
output of a given G-linked receptor in a cell, said
method comprising introducing into the cell a chimeric
polypeptide comprising:
(a) a first polypeptide having the contiguous
25 sequence of a 4 or 5 residue C-terminal segment of a
first $G\alpha$ subunit, wherein said first $G\alpha$ subunit is a $G\alpha$
subunit to which said receptor naturally links; and
(b) a second polypeptide having the entire, except
for 4 or 5 C-terminal residues, contiguous sequence of a
30 second $G\alpha$ subunit, wherein said second $G\alpha$ subunit, when
activated, leads to a signal-transducing output different
from that of said first $G\alpha$ subunit;

provided that (1) if said first Gα subunit is $G\alpha_i$, said second Gα subunit cannot be $G\alpha_q$; and (2) if said first Gα subunit is $G\alpha_{13}$, said second Gα subunit cannot be $G\alpha_z$.

5   16.   The method of claim 15, wherein said receptor is APP, Ile-APP, Phe-APP, or Gly-APP; said first Gα subunit is $G\alpha_o$; and said second Gα subunit is $G\alpha_s$.

17.   The method of claim 16, wherein the cell is a neural cell of a mammal.

10   18.   The method of claim 17, wherein said mammal is an Alzheimer's Disease patient.

19.   A nucleic acid molecule comprising a promoter operably linked to a sequence encoding a chimeric polypeptide comprising (a) amino acid residues 1-389 of

15   SEQ ID NO:21, and (b) an amino acid sequence representing the C-terminal 5 residues of a naturally occurring Gα polypeptide that is not $G\alpha_s$.

20.   The nucleic acid molecule of claim 19, wherein said C-terminal 5 residues are selected from the

20   group consisting of SEQ ID NOs:22-30.

21.   A method of inhibiting the growth of a tumor cell, said method comprising:
(1) introducing into the tumor cell (a) a somatostatin receptor type 5 polypeptide, or (b) a

25   nucleic acid molecule that directs the expression of said polypeptide in the cell; and
(2) contacting the cell with somatostatin or a biologically active analogue of somatostatin.

22. The method of claim 21, wherein said tumor cell is a human small cell lung cancer cell.

23. The method of claim 22, wherein said nucleic acid molecule comprises a viral vector.

5   24. The method of claim 21, said method comprising the additional step of, prior to said contacting step, introducing into the tumor cell (1) a $G\alpha_{12}$ or $G\alpha_{13}$ polypeptide, or (2) a nucleic acid molecule that directs the expression of said $G\alpha_{12}$ or $G\alpha_{13}$
10   polypeptide in the cell.

25. A method of inhibiting the growth of a tumor cell, the growth of which is stimulated via an endogenous, hyperactive G-linked receptor, said method comprising introducing into said tumor cell a polypeptide
15   comprising   (1) an amino acid sequence representing the C-terminal 4 or 5 contiguous residues of a $G\alpha$ that naturally couples to said G-linked receptor; and
     (2) an amino acid sequence representing the entire, except the C-terminal 4 or 5 residues, contiguous
20   sequence of $G\alpha_{12}$ or $G\alpha_{13}$.

26. A nucleic acid molecule comprising a promoter operably linked to a sequence encoding a chimeric polypeptide comprising:
     (1) an amino acid sequence representing the C-
25   terminal 4 or 5 residues of a first $G\alpha$ subunit;
     (2) an amino acid sequence representing all except the C-terminal 4 or 5 residues of a second $G\alpha$ subunit, said second $G\alpha$ subunit being $G\alpha_{12}$ or $G\alpha_{13}$;
     provided that when said first $G\alpha$ subunit is $G\alpha_{z}$,
30   said second $G\alpha$ subunit cannot be $G\alpha_{13}$.

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27.   A polypeptide the amino acid sequence of which comprises:

(1) a sequence representing the C-terminal 4 or 5 contiguous residues of a first $G\alpha$ subunit that naturally
5   couples to said G-linked receptor;

(2) a sequence representing all except the C-terminal 4 or 5 residues of a second $G\alpha$ subunit, said second $G\alpha$ subunit being $G\alpha_{12}$ or $G\alpha_{13}$;

provided that when said first $G\alpha$ subunit is $G\alpha_z$,
10   said second $G\alpha$ subunit cannot be $G\alpha_{13}$.

N		DONOR OF C TERMINUS		
Gαs wt	Gαs	QYELL	Gαs	[SEQ ID NO:31]
Gαs/αi1	Gαs	DCGLF	Gαi1(i2)	[SEQ ID NO:22]
Gαs/αi3	Gαs	ECGLY	Gαi3	[SEQ ID NO:23]
Gαs/αo	Gαs	GCGLY	Gαo1(o2)	[SEQ ID NO:24]
Gαs/αz	Gαs	YIGLC	Gαz	[SEQ ID NO:25]
Gαs/αq	Gαs	EYNLV	Gαq(11)	[SEQ ID NO:26]
Gαs/α12	Gαs	DIMLQ	Gα12	[SEQ ID NO:27]
Gαs/α13	Gαs	QLMLE	Gα13	[SEQ ID NO:28]
Gαs/α14	Gαs	EFNLV	Gα14	[SEQ ID NO:29]
Gαs/α16	Gαs	EINLL	Gα16	[SEQ ID NO:30]

389 390 394

1

FIG. I
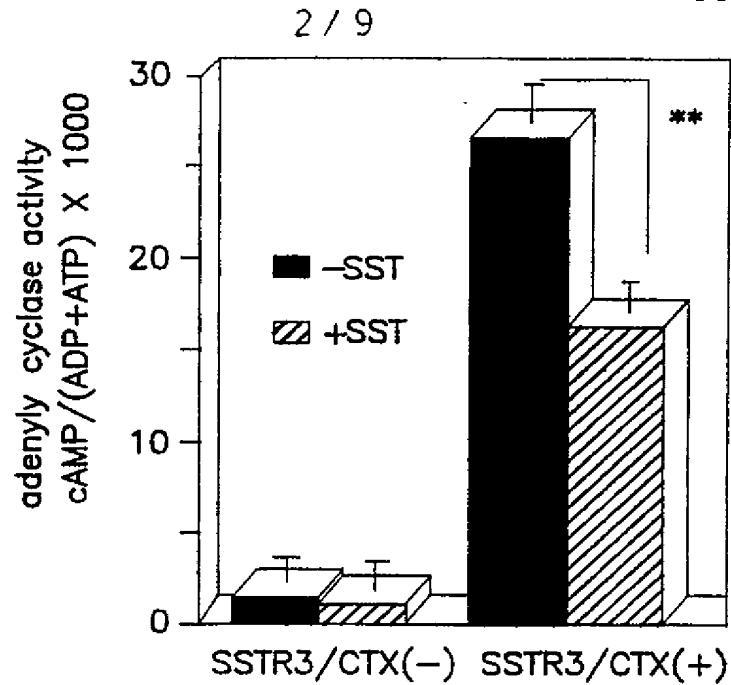
FIG. 2A
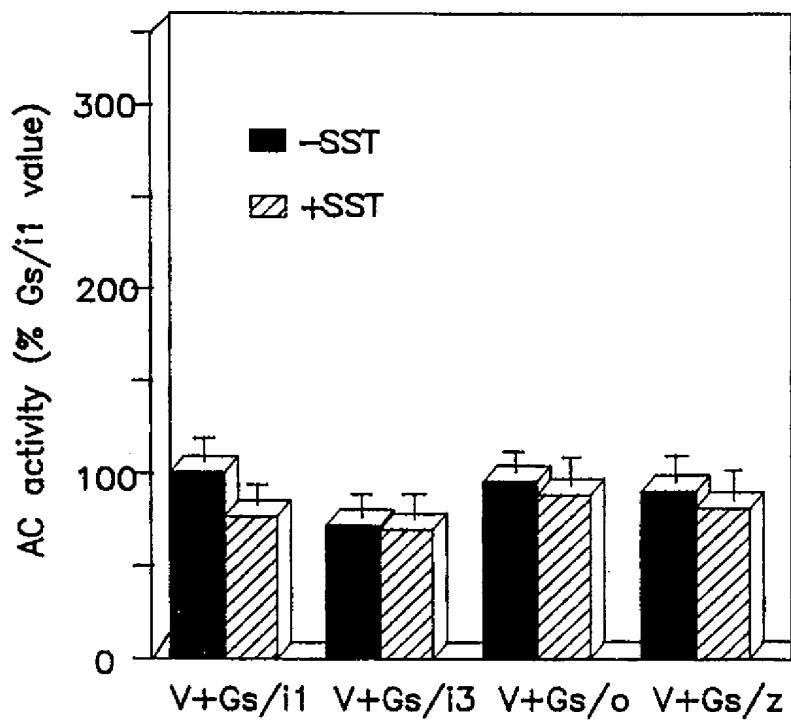
FIG. 2B
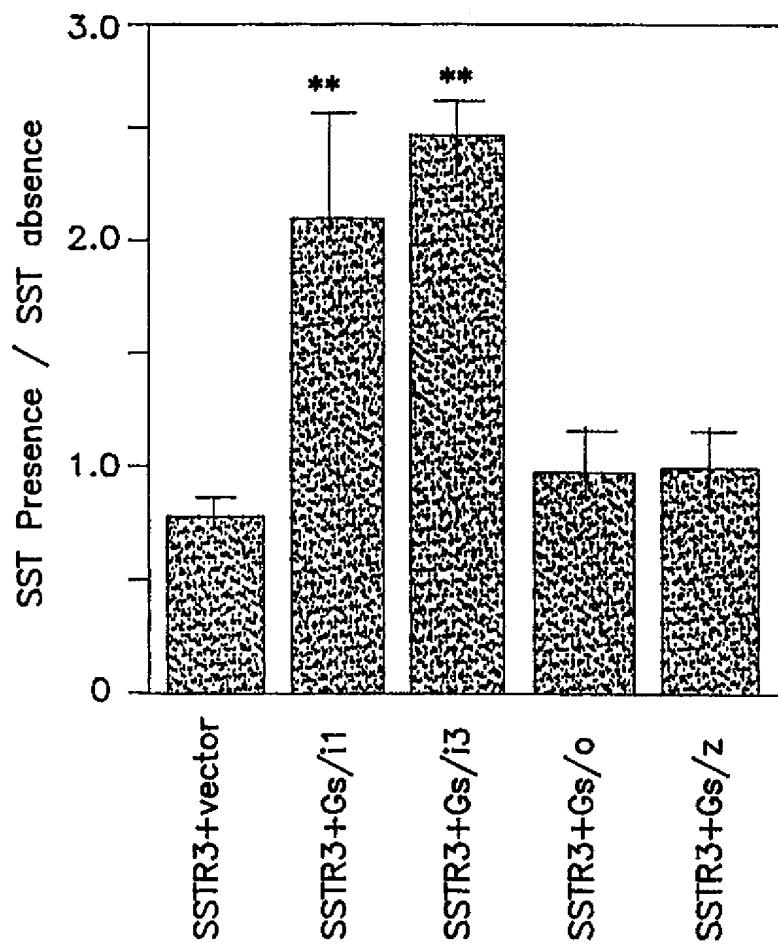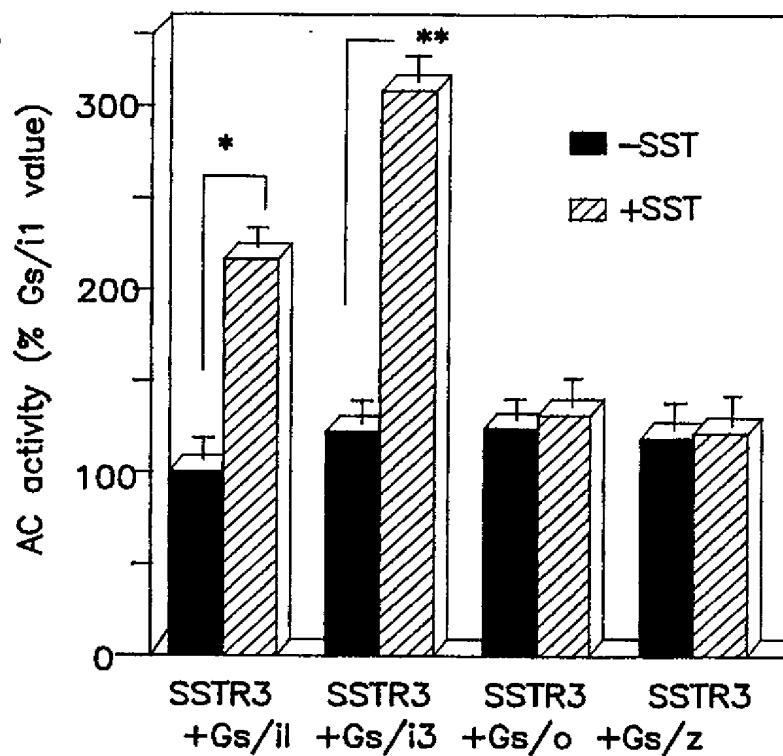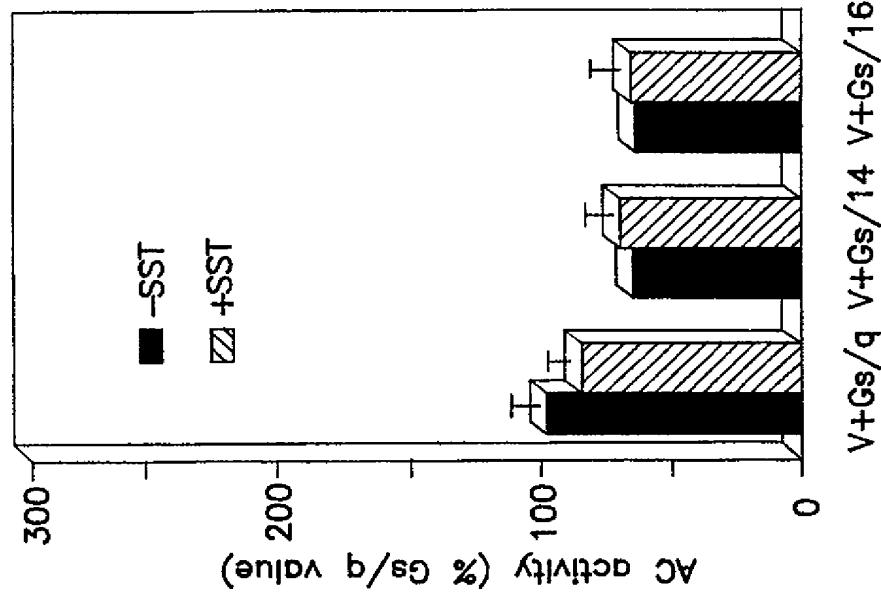
FIG. 2C

FIG. 2D

FIG. 3B

FIG. 3A

FIG. 3D

FIG. 3C

NSDOCID: <WO_____9816557A1_I_>
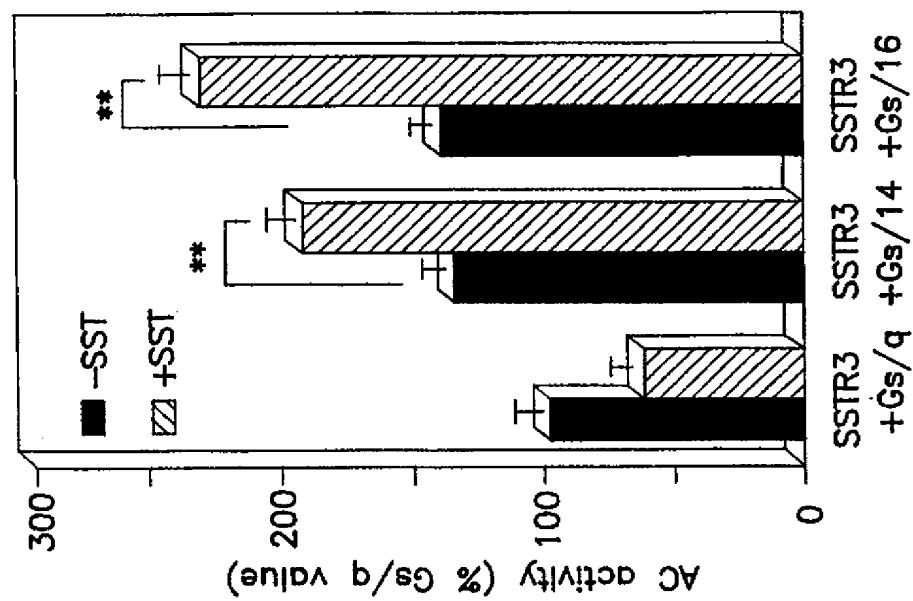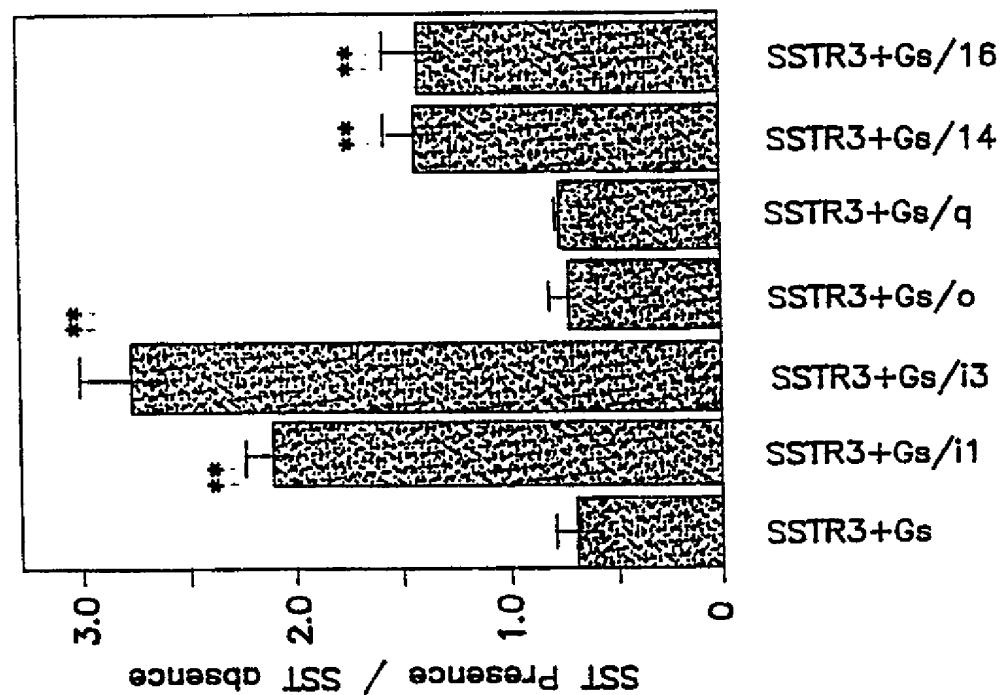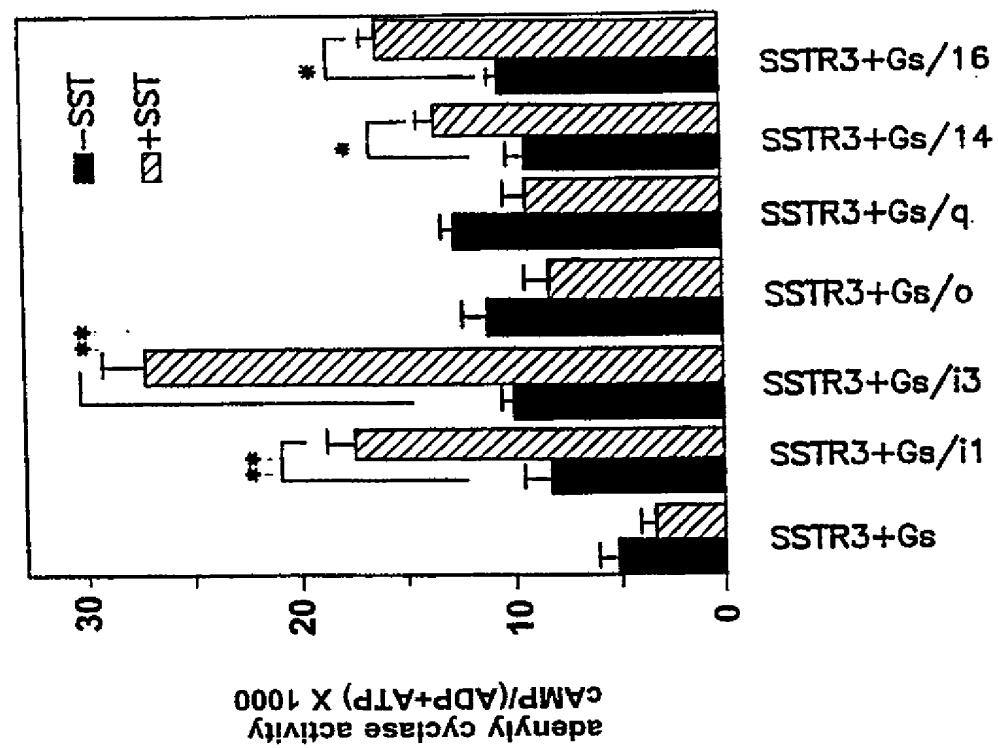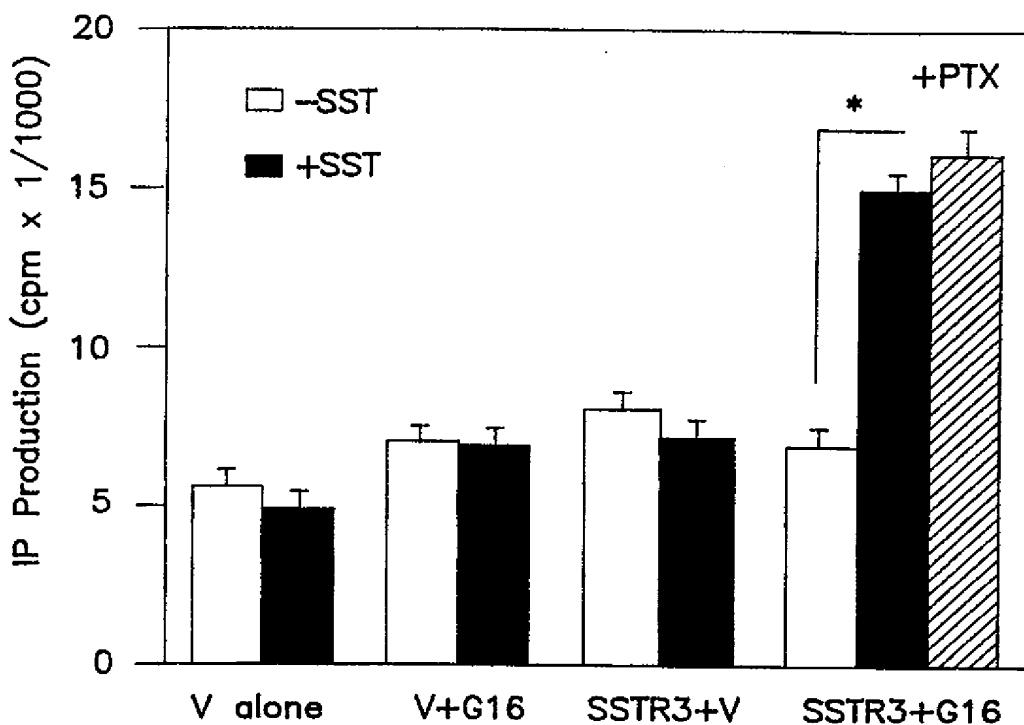
FIG. 4A
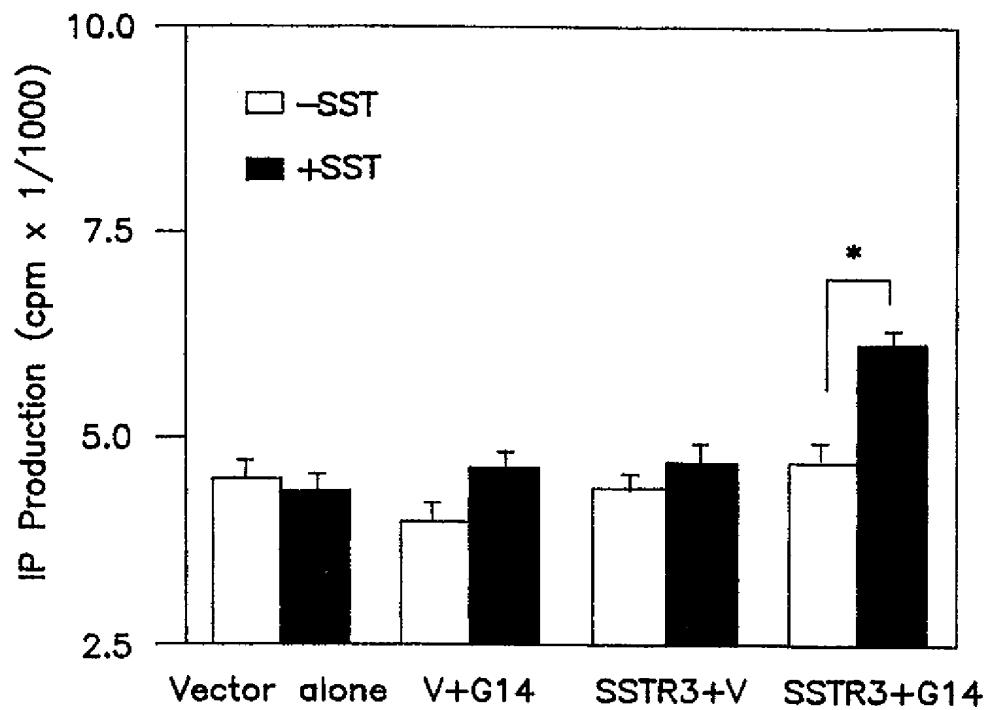
FIG. 4B
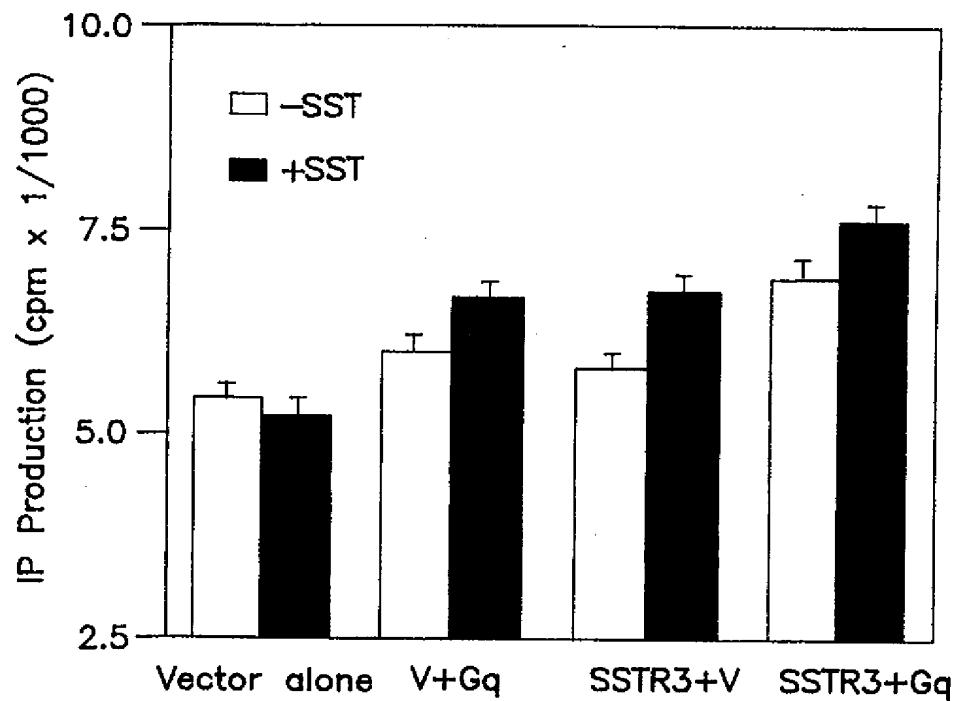
FIG. 4C
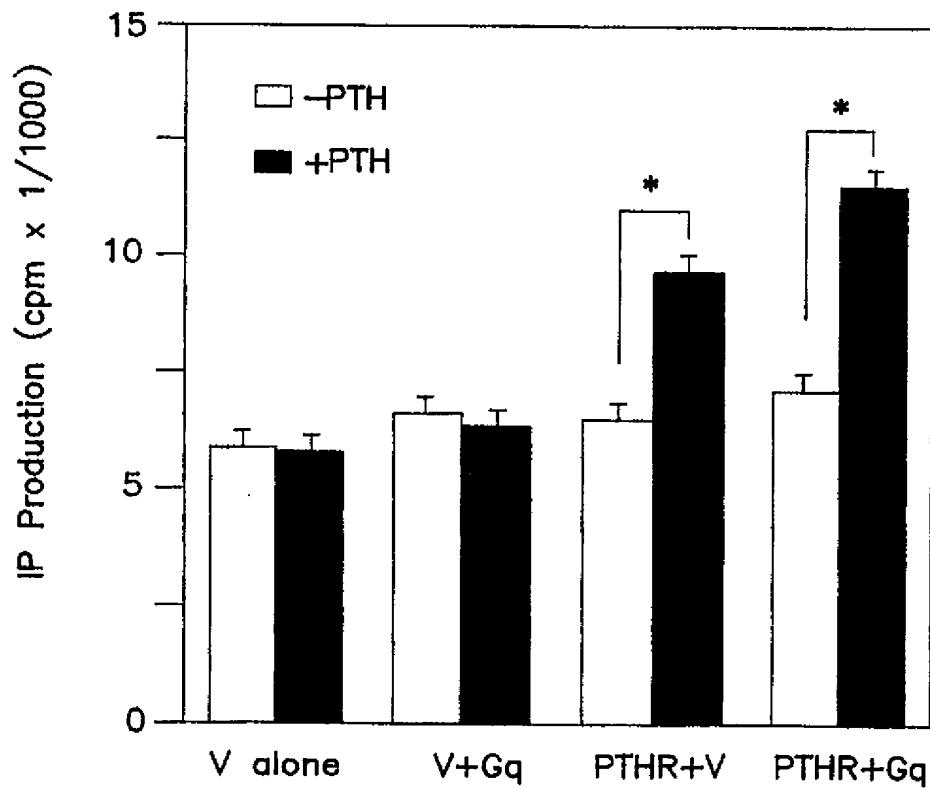
FIG. 4D
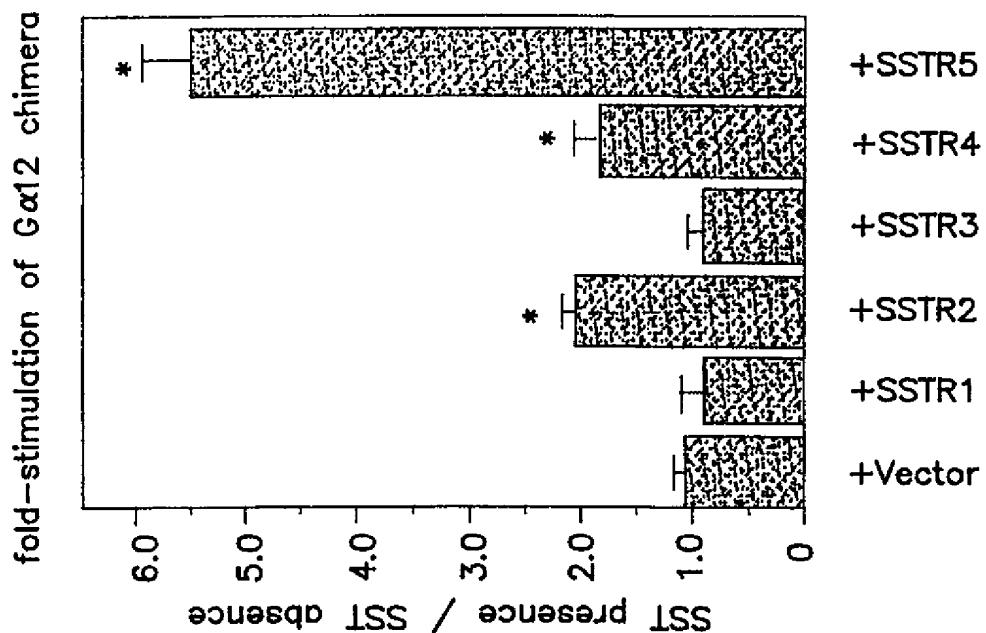
FIG. 5B
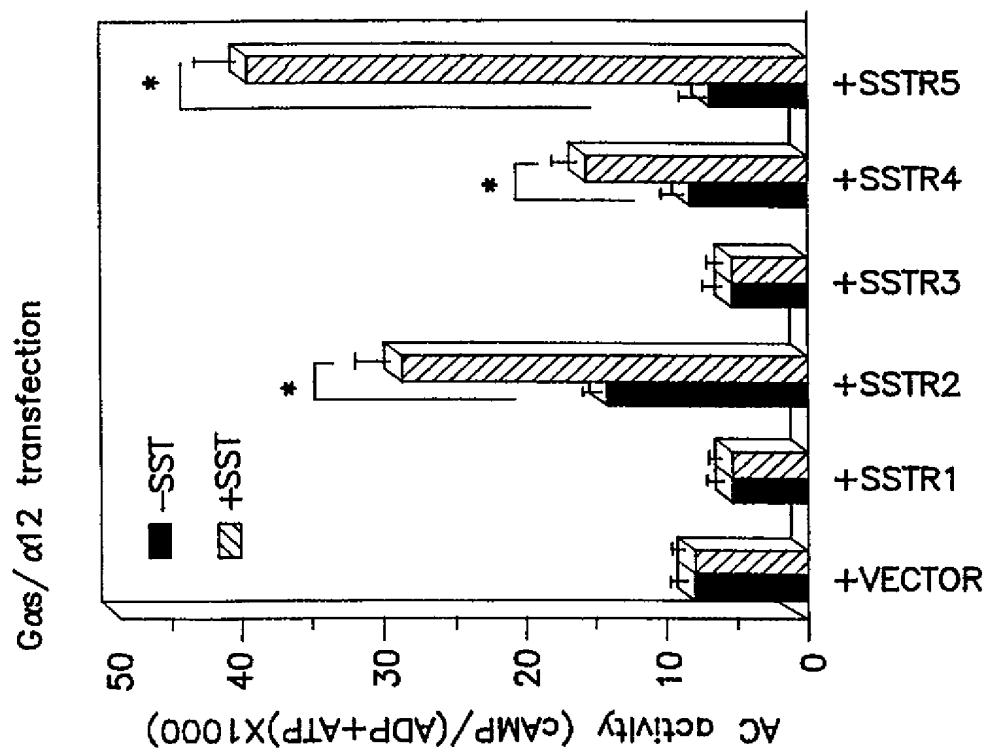
FIG. 5A
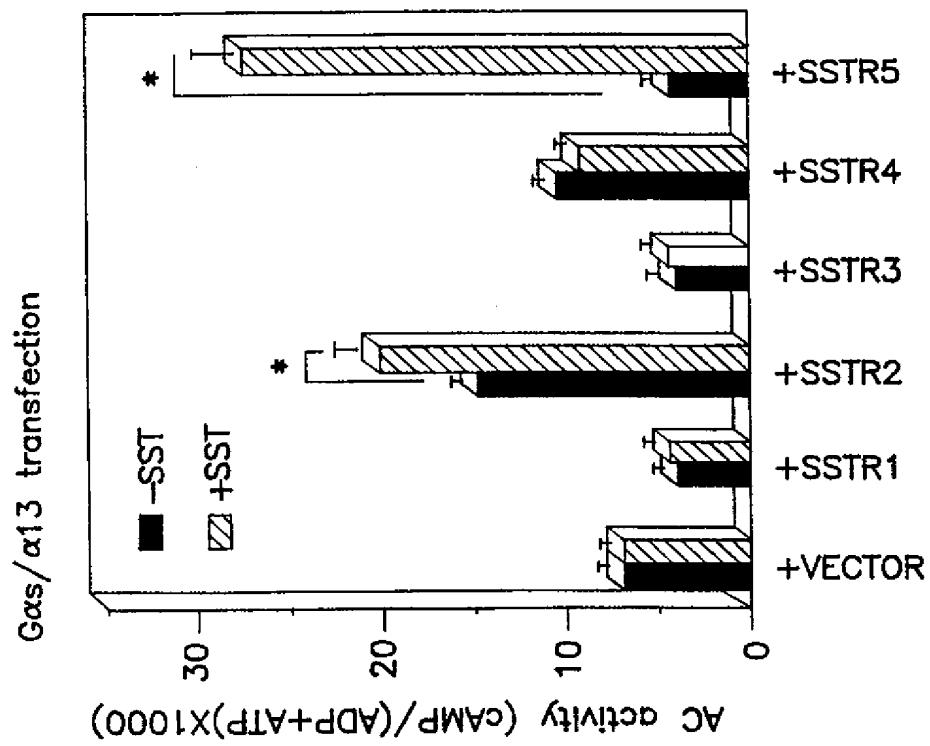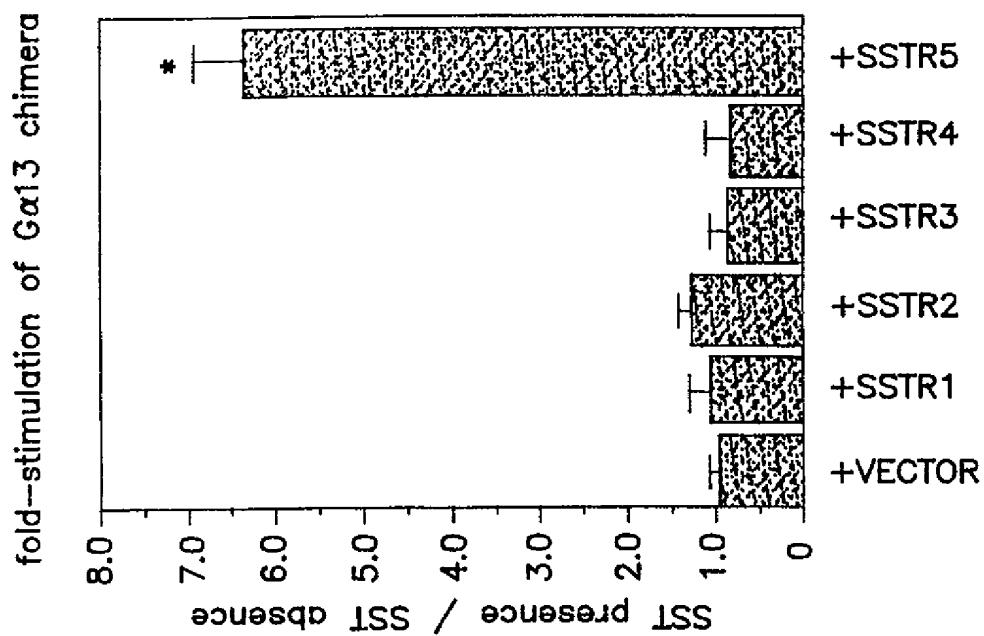
FIG. 5D

FIG. 5C

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6)   :C07K 14/705; C12N 15/12, 15/62; C12Q 1/02; G01N 33/53

US CL   :435/ 7.1, 29, 69.7; 530/350; 536/23.4.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. :   435/ 7.1, 29, 69.7; 530/350; 536/23.4.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
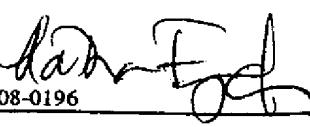
Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y ----- A	BERLOT, C.H. et al. Identification of effector-activating residues of $G_{s\alpha}$. Cell. 06 March 1992, Vol. 68, pages 911-922, especially page 911, 912, 919, and 920.	1-7, 9-20, 26, 27 ----- 8
Y ----- A	CONKLIN, B.R. et al. Substitution of three amino acids switches receptor specificity of $G_q\alpha$ to that of $G_i\alpha$. Nature. 20 May 1993, Vol. 363, pages 274-276, especially page 274, figure 1.	1-7, 9-20, 26, 27 ----- 8
Y ----- A	VOYNO-YASENETSKAYA, T. et al. $G\alpha13$ stimulates Na-H exchange. J. Biol. Chem. 18 February 1994, Vol. 269, No. 7, pages 4721-4724, especially page 4723, figure 3.	26, 27 ----- 1-20

☒ Further documents are listed in the continuation of Box C.   ☐ See patent family annex.

*	Special categories of cited documents:	
"A"	document defining the general state of the art which is not considered to be of particular relevance	
"E"	earlier document published on or after the international filing date	
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	
"O"	document referring to an oral disclosure, use, exhibition or other means	
"P"	document published prior to the international filing date but later than the priority date claimed	

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 FEBRUARY 1997	0 1 APR 1997

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	MICHAEL D. PAK
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

International application No.

PCT/US96/20510

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y ----- A	LAW, S.F. et al. $G_{i\alpha 1}$ selectively couples somatostatin receptor subtype 3 to adenylyl cyclase: identification of the functional domains of this α subunit necessary for mediating the inhibition by somatostatin of cAMP formation. Molecular Pharmacology. April 1994, Vol. 45, No. 4, pages 587-590, especially page 589, figures 2 and 3.	1-7, 9-20, 26, 27 ----- 8
Y ------ A	US 5,559,209 A (NISHIMOTO) 24 September 1996 (24.09.96), especially columns 1-3.	1-7, 9-18 ----- 8
Y, P	US 5,578,451 A (NISHIMOTO) 26 November 1996 (26.11.96), especially columns 1-4.	10, 12-18
Y	YAMATSUJI, T. et al. G protein-mediated neuronal DNA fragmentation induced by familial Alzheimer's disease-associated mutants of APP. Science. 31 May 1996, Vol. 272, pages 1349-1352, especially pages 1349 and 1351.	10, 12-18

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/20510

Box I   Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐   Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐   Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such
   an extent that no meaningful international search can be carried out, specifically:

3. ☐   Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II   Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

   Please See Extra Sheet.

1. ☐   As all required additional search fees were timely paid by the applicant, this international search report covers all searchable
   claims.

2. ☐   As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
   of any additional fee.

3. ☐   As only some of the required additional search fees were timely paid by the applicant, this international search report covers
   only those claims for which fees were paid, specifically claims Nos.:

4. ☒   No required additional search fees were timely paid by the applicant.   Consequently, this international search report is
   restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
   1-20, 26, 27

Remark on Protest   ☐   The additional search fees were accompanied by the applicant's protest.

☐   No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet(1))(July 1992)★

BNSDOCID: ≺WO   0016557A1 I ≻

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, STN-BIOSCIENCE, CAPLUS, MEDLINE, BIOSIS, EMBASE, SCISEARCH, SWISS-PROT, A-GENESEQ, PIR.

search terms: g-protein, chimer?, c-termin?

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-19, 20, 26, and 27, drawn to a method of determining whether a given G-linked receptor associates with the G-alpha subunit, a method of identifying a compound that can modulate the interaction between a given G-linked receptor associates with the G-alpha subunit, a method of identifying a compound that can modulate the interaction between a G-alpha-o and a constitutively active mutant of APP, a method of altering the signal-transducing output of a given G-linked receptor in a cell, a nucleic acid molecule, and polypeptide..

Group II, claims 21-24, drawn to a method of inhibiting the growth of a tumor cell with somatostatin.

Group III, claim 25, drawn to a second method of inhibiting the growth of a tumor cell.

The inventions listed as Groups I-III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons.

     The special technical feature of Group I is a method using the specific G-protein, a nucleic acid, and polypeptide. Pursuant 37 CFR 1.475(d), these claims are considered by the ISA/US to constitute the main invention, and none of the related groups II-III correspond to the main invention.
     The product of Group I does not share the same special technical feature as the methods of groups II and III because the product of group I is not used in or produced by the methods of groups II and III, and each defines a separate invention over the art.
     The methods of Groups I, II, and III, do not share a special technical feature in any pairing because the methods have materially different process steps and are practiced for materially different purposes, and each defines a separate invention over the art.
     Since no special technical feature of any group other than the main invention is shared by any of the other inventions, unity of invention is lacking.

WO_____9816557A1_I_>